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Alternativní reprodukční strategie a pohlavní výběr u vlaštovky obecné *Hirundo rustica*

Alternative reproductive strategies and sexual selection in barn swallow *Hirundo rustica*

Disertační práce

Školitel: prof. Tomáš Albrecht, Ph.D.

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Titul disertační práce: Alternativní reprodukční strategie a pohlavní výběr u vlaštovky obecné *Hirundo rustica*

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(1)

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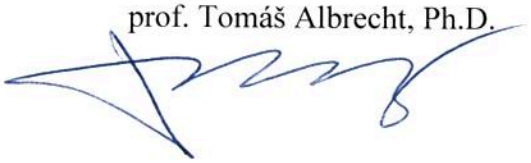

(2)

Tomášek O., Pap P. L., Adámková M., Cepák J., Fülöp A., **Michálková R.**, Stermin A. N., Vágási C. I., Vincze O. Albrecht T. Age-dependence and viability signalling function of tail streamer length in European barn swallow (*in preparation for Journal of Animal Ecology*).

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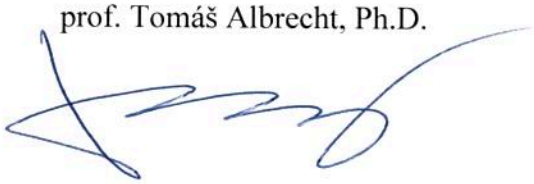

(3)

Pap P. L., Fülöp A., Adámková M., Cepák J., **Michálková R.**, Safran R. J., Stermin A. N., Tomášek O., Vágási C. I., Vincze O., Wilkins M. R. & Albrecht T. (2019). Selection on multiple sexual signals in two Central and Eastern European populations of the barn swallow. *Ecology and Evolution* 9: 11277-11287.

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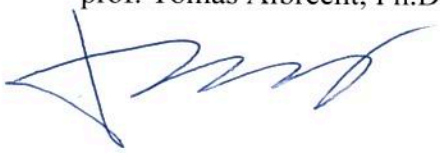

(4)

Michálková R., Tomášek O., Adámková M., Kreisinger J. & Albrecht T. (2019). Extra-pair paternity patterns in European barn swallows *Hirundo rustica* are best explained by male and female age rather than male ornamentation. *Behavioral Ecology and Sociobiology* 73: 119.

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prof. Tomáš Albrecht, Ph.D.

OBSAH

ABSTRAKT:

Rozvoj molekulárních technik odhalil, že párovací systém u ptáků je mnohem složitější a komplexnější, než bylo původně předpokládáno. Jak samci, tak samice mohou využívat alternativní rozmnožovací strategie ke zvýšení svého vlastního reprodukčního úspěchu. V předložené práci jsme zdokumentovali výskyt hnízdního parazitismu (conspecific brood parasitism, CBP, 22 % hnízd), quasi-parazitismu (QP, 6.5 % hnízd) a mimo-párových paternit (extra-pair paternity, EPP, 51.2 % hnízd) u evropského poddruhu vlaštovky obecné (*Hirundo rustica rustica*). Na rozdíl od EPP a CBP, je quasi-parazitismus poměrně vzácný a byl popsán jen u několika druhů. Naše výsledky naznačují nenáhodnou distribuci QP, což z něj může činit, vedle EPP a CBP, třetí alternativní reprodukční strategii.

Samčí ornamenty se pravděpodobně vyvinuly jako kondičně-závislé signály kvality svého nositele. V daném kontextu by tedy měla exprese takových znaků pozitivně korelovat s přežíváním jedince. Evoluční teorie předpokládá, že samice si k mimo-párovým kopulacím vybírají kvalitnější (více ornamentované) samce a mimo-párové paternity byly následně navrženy jedním z možných protagonistů v evoluci samčích pohlavních znaků. Evidence pro podporu tohoto tvrzení je však poměrně slabá. I přes intenzivní výzkum role EPP v pohlavním výběru se stále diskutuje o potenciálních výhodách z promiskuitního chování pro samice a relativně málo studií dokumentuje, že by mimo-párové paternity vytvářely selekční tlak na samčí ornamenty. Robustním prediktorem úspěchu v mimo-párových paternitách se u ptáků zdá být samčí věk. Exprese ornamentů je často asociována se stářím jedince a může proto odrážet jeho životaschopnost.

V této práci jsme testovali signalizační funkci prodloužených rýdovacích per u vlaštovky obecné, učebnicového příkladu druhotného pohlavního ornamentu, a jejich vliv na reprodukční úspěšnost samce. Délka ocasních per se prodlužovala s věkem u obou pohlaví, samci s delšími ocasy navíc vykazovali lepší přežívání, což podporuje funkci daného ornamentu jako signálu životaschopnosti jedince. Experimentální manipulace délky ocasních per však neměla žádný efekt na přežívání. To může indikovat spíše nízké náklady spojené s expresí tohoto znaku. Dále jsme zdokumentovali mezi-populační rozdíly v míře exprese ornamentů (délka ocasních per a ventrální zbarvení těla) mezi rumunskou a českou populací studovaného druhu. Rozdíly byly detekovány také mezi pohlavím a věkovými třídami v rámci jedné populace.

Na rozdíl od předchozích studií nenaznačují naše výsledky, že by délka ocasních per měla hlavní vliv na fertilizační úspěch samce (ani vnitro-párový, ani mimo-párový). Nejsilnějším prediktorem samčí i samičí promiskuity je v námi studované české populaci vlaštovky obecné věk samce, respektive samice. Oddělení efektu věku a exprese ornamentu se zdá být zásadním krokem při zkoumání vlivu EPP na evoluci pohlavně selektovaných znaků u ptáků.

ABSTRACT:

Molecular techniques have revealed that avian mating system is more diverse and complex than previously thought. Both males and females can use alternative reproductive tactics to increase their fitness. Here, we have determined the prevalence of conspecific brood parasitism (CBP, 22% of nests), quasi-parasitism (QP, 6.5% of nests) and extra-pair paternity (EPP, 51.2% of nests) in European subspecies of barn swallow (*Hirundo rustica rustica*). In contrast to EPP and CBP, QP is rare and has been described in only a few bird species. Our data indicate nonrandom QP patterns, suggesting that this tactic can be considered a third alternative reproductive strategy, a longside CBP and EPP.

Sexual ornaments are usually assumed to evolve as condition-dependent signals of individual quality. In this context, a positive correlation between the expression of ornamental traits and survival, is expected. Evolutionary theory predicts that females seek extra-pair fertilizations from high-quality (more ornamented) males and EPP has consequently been proposed as a principal protagonist in the evolution of male secondary sexual traits, however empirical evidence in support of the assertion is relatively scarce. Despite decades of research into the role of EPP in sexual selection, the potential fitness advantages for females pursuing EPP are still debated. Notably, relatively few studies have shown that EPP creates directional selection pressures on male ornaments. In birds, male age is a robust predictor of success in extra-pair paternity. Ornament expression is often associated with age and thus can reflect an individual quality.

In this thesis, we have tested the viability signalling function of elongated tail streamers, the textbook example of a sexually selected trait, and their effect on within-pair and extra-pair success in barn swallows. Tail length increased with age in both sexes, moreover, we detected a positive correlation between tail length and lifespan in males, which support the viability signalling function of this trait. Experimental manipulation of tail streamers length, however, showed no effect on survival, suggesting a generally low cost of this ornament. We also documented inter-population differences in the expression of ornaments (length of tail streamers and ventral coloration) between two populations of barn swallow (the Czech Republic and Romania). Differences were also detected between the sexes and age classes within the population.

In contrast to previous studies, our data did not provide support for tail streamer length being a strong predictor of fertilization success (neither within-pair nor extra-pair) in our Czech population. We identified age as the major predictor of male and female promiscuity. The separation of the effects of ornament expression and male age seems to be a crucial step in assessing the role of EPP in the evolution of male secondary sexual traits in birds.

ÚVOD A SYNTÉZA

Alternativní reprodukční strategie

Studium reprodukčních strategií patří k zásadním tématům evoluční biologie již po 150 let (Darwin 1871). S rozvojem molekulárních metod určování rodičovství a příbuznosti jedinců došlo k revoluci našeho chápání a nahlížení na danou problematiku.

Monogamie byla dříve považována za nejrozšířenější způsob rozmnožování v ptačí říši. Její definice vychází z předpokladu, že sociální svazek uzavřený mezi jedním samcem a jednou samicí vede ke zplození společných potomků, o které se oba jedinci v páru následně starají (Lack 1968). Je ovšem potřeba rozlišovat mezi monogamií genetickou, při níž oba rodiče pečují o své skutečné biologické potomky, a monogamií sociální, tedy situací, ve které jak samec, tak samice investují do péče o potomstvo, ačkoliv ne všechna mláďata jsou jejich skutečnými, genetickými potomky (Griffith *et al.* 2002). Právě sociální monogamie je nejběžnějším párovacím systémem u ptáků (Griffith *et al.* 2002; Brouwer & Griffith 2019). U mnoha sociálně monogamních druhů byl však odhalen výskyt komplexních reprodukčních taktik, které mohou vést ke vzniku mimo-párového potomstva (Wrege & Emlen 1987). Takovými taktikami mohou být (1) vnitrodruhový hnízdní parazitismus (conspecific brood parasitism – CBP), (2) quasi-parazitismus (quasi-parasistism – QP) a/nebo (3) mimo-párové paternity (extra-pair paternity – EPP).

(1) Vnitrodruhový hnízdní parazitismus (CBP)

K hnízdnímu parazitismu dochází v případě, kdy parazitická samice snese vejce do hnízda jiné, hostitelské, samice bez následné rodičovské péče (Payne 1977; Davies 2000; Yom-Tov 2001). Obecně lze rozlišovat mezi parazitismem obligátním, tedy takovým, kdy je parazitování jedinou možností reprodukce pro daný druh, a parazitismem fakultativním, tedy příležitostným, při kterém samice snáší některá vejce paraziticky, ale zároveň může založit vlastní snůšku (Yom-Tov & Geffen 2006). Hnízdní parazitismus je možné dále rozdělit na úrovni druhu, který se stal hostitelem pro parazitickou samici na (1) mezidruhový (interspecifický – samice parazituje jiný druh) a (2) vnitrodruhový (konspecifický – samice snáší vejce do jiného hnízda stejného druhu), který se vyskytuje pouze u fakultativního typu parazitace (Davies 2000).

Ve všech případech je péče o mimo-párová mláďata nákladná pro oba hostitelské rodiče, uvažujeme-li druh s biparentální péčí (Petrie & Møller 1991). Právě péče o potomstvo je zásadní faktor uplatňující se v evoluci této reprodukční strategie. Není proto překvapivé, že

hnízdní parazitismus se objevuje častěji u druhů s nižšími nároky na rodičovskou péči, tedy u druhů prekociálních, jako např. u vrubozobých *Anseriformes* (Yom-Tov 1980; Sorenson 1992; Eadie *et al.* 1989, Yom-Tov 2001). U druhů s altriciálním módem péče o mláďata, u kterých je nutná intenzivní péče rodičů nejen v době inkubace ale také v době krmení mláďat, je vnitrodruhový hnízdní parazitismus obecně málo rozšířen (Yom-Tov 2001; Yom-Tov & Geffen 2006).

Mnoho prací, zabývající se CBP, se však potýkalo s problémem správného určení parazitické události, které bylo, do doby využití molekulárně-genetických přístupů, poměrně problematické. Metody často využívané k detekci parazitického vejce, jako např. abnormální velikost snůšky, přítomnost dvou a více vajec v hnízdě během jednoho dne nebo rozdílná velikost, barva či vzor daného vejce, mohou výskyt a frekvenci vnitrodruhového parazitismu v populaci zkreslovat (Andersson & Åhlund 2001; Griffith *et al.* 2009; **Publikace 1**).

Existuje několik hypotéz, snažících se vysvětlit adaptivní funkci CBP. První navrhuje, že k parazitismu dochází v důsledku nevhodných, či ne úplně optimálních podmínek pro klasické hnízdění, ať už se jedná o limitaci prostředí či sníženou kondici dané samice („best-of-a-bad-job strategy“, Dawkins 1980). Alternativní hypotéza předpokládá, že parazitické chování vede ke zvýšení reprodukčního úspěchu samice („enhancement hypothesis“, Kendra *et al.* 1988). Parazitické samice lze rozdělit do tří skupin: (1) samice, které ztratily vlastní snůšku v důsledku predace (Shaw & Hauber 2009), (2) nespárované samice, obvykle bez zkušenosti s hnízděním, které nemají vlastní hnízdo a parazitismus je pro ně možnost získat alespoň nějaký reprodukční úspěch, (3) spárované samice, tzv. „hnízdící parazité“, které mimo vlastní hnízdní pokus kladou některá vejce paraziticky, čímž si zvyšují reprodukční úspěch (Yom-Tov 1980). Samice, které využívají tuto strategii hnízdících parazitů, mohou svůj reprodukční úspěch až zdvojnásobit (Andersson & Åhlund 2001). Navíc tím snižují kompetici mezi mláďaty v jedné snůšce, a zároveň rozmělňují riziko mortality všech mláďat v případě predace celé snůšky v jednom hnízdě (Ruxton & Broom 2002, Andersson & Åhlund 2012).

Poněvadž rodičovská péče je energeticky nákladná (Cluttton-Brock 1991), snižuje CBP fitness obou pěstounských rodičů, a to hned několika způsoby: (1) hostitelská snůška může být redukována v důsledku zničení či odstranění vajec parazitickou samicí během parazitické události (Lombardo *et al.* 1989), (2) zvětšená hostitelská snůška může být zatížena vyšší pravděpodobností predace v důsledku lepší viditelnosti a delší doby inkubace (Gibbons 1986; Nielsen *et al.* 2006), (3) parazitovaná snůška může mít sníženou líhnivost (Eadie 1989; Semel & Sherman 2001), (4) prodloužená doba inkubace může dále vést ke snížení reziduálních

žloutkových zásob u vylíhlých mláďat, a tím negativně ovlivňovat jejich růst a přežívání (Hepp *et al.* 1990), (5) hostitelští rodiče mohou trpět nižší mírou přežívání (Brown & Brown 1991).

Dalo by se tedy očekávat, že oba jedinci daného sociálního páru budou chránit hnízdo před nechtěnou parazitickou samicí (Petrie & Møller 1991). Nicméně existují případy, kdy sociální pár nebo jeden ze sociálních rodičů, akceptuje parazitické chování jiné samice, což lze vysvětlit několika způsoby. Sociální samec může zredukovat agresi vůči parazitické samici v případě, že tato samice s ním bude následně kopulovat (Petrie 1986; Lyon *et al.* 2002). Další možností snížené agrese může být genetická příbuznost parazitické samice s jedním ze sociálních rodičů parazitovaného hnízda (Andersson 1984; Andersson & Åhlund 2000; Andersson *et al.* 2019). V takové situaci jsou totiž náklady spojené s péčí o parazitické mládě kompenzovány inkluzivní fitness alespoň jednoho ze sociálních rodičů (Lyon & Eadie 2008) a kin selekce tak může usnadňovat evoluci CBP (Andersson 1984). Poslední vysvětlení je založené na možnosti kooperace hostitelského samce s parazitickou samicí (Emlen & Wrege 1986), tedy na výskytu tzv. quasi-parazitismu.

(2) Quasi-parazitismus (QP)

Kromě vnitrodruhového hnízdního parazitismu může k mimo-párové maternitě docházet v důsledku quasi-parazitismu. Ten nastává, pokud parazitická samice snese vejce do hnízda jiné samice, přičemž toto vejce je oplodněno sociálním partnerem parazitované samice (Emlen & Wrege 1986; Griffith *et al.* 2004). V populaci, ve které dochází k hnízdnímu parazitismu, a zároveň ke genetické promiskuitě, existuje určitá pravděpodobnost, že samice oplodněná při mimo-párových kopulacích snese vejce do hnízda mimo-párového samce, formou parazitace, náhodou (Alves & Bryant 1998). Odlišná situace nastává, pokud samice snese vejce fertilizované mimo-párovým samcem do hnízda stejného samce záměrně. Nenáhodná distribuce quasi-parazitismu v populaci z něj může činit adaptivní strategii (Alves & Bryant 1998; Berger *et al.* 2014; **Publikace 1**). V případě této reprodukční taktiky trpí zvýšenými nároky na rodičovskou péči o cizí mládě pouze hostitelská samice, nikoliv její partner (Lyon & Eadie 2008). Ten může naopak takovým chováním zvyšovat svůj reprodukční úspěch a kooperace s parazitickou samicí pro něj tudíž může být výhodná (**Publikace 1**). Zatímco je vnitrodruhový hnízdní parazitismus často dokumentovaný jevem, evidence quasi-parazitismu zůstává dosud poměrně vzácná, zejména u druhů s altriciálním modelem rodičovské péče. Vzácnost daného chování není zcela překvapující, vezmeme-li v úvahu soubor relativně složitých a neobvyklých podmínek, které jsou pro adaptivní vývoj QP potřebné (Griffith *et al.*

2004). Evolučně-ekologické konsekvence quasi-parazitismu nejsou zcela objasněny a studium tohoto vzácného fenoménu vyžaduje větší pozornost.

(3) Mimo-párové paternity (EPP)

Nejvíce prostudovanou reprodukční strategií, kterou využívá více než 75 % ptačích druhů, jsou mimo-párové paternity (Griffith *et al.* 2002; Westneat & Stewart 2003, Brouwer & Griffith 2019). Hovoříme o nich v případě, kdy spárovaný samec kopuluje se samicí jinou, než je jeho sociální partnerka (Westneat *et al.* 1990). Pokud taková kopulace vede k oplození, výsledkem jsou mimo-párová mláďata v hnízdě samice, účastnící se páření mimo svazek, a jejího sociálního partnera. Zatímco se samčí zájem o mimo-párové kopulace zdá být intuitivní, ve smyslu zvýšeného množství zplozených potomků bez nutnosti následné rodičovské péče (Trivers 1972; Wakano & Ihara 2005), samičí motivace k účasti v kopulacích s více samci není natolik zřejmá (Akçay & Roughgarden 2007, Mays *et al.* 2008), a otázka, zda je tato taktika řízena samčím nebo samičím zájmem, zůstává nezodpovězena (Eliassen & Kokko 2008).

Mezi jednotlivými druhy navíc existuje značná variabilita v míře EPP – zatímco u některých druhů nebyla mimo-párová paternita dokumentována, u jiných druhů dosahuje míry až téměř 90 % hnízd v populaci (Dixon *et al.* 1994; Griffith *et al.* 2002; Lifjeld *et al.* 2019). Ačkoliv bylo navrženo a následně testováno několik možných hypotéz, snažících se vysvětlit tuto variabilitu (Petrie & Kempenaers 1998; Griffith *et al.* 2002; Arnold & Owens 2002; Bennett & Owens 2002), důvod, proč je promiskuita mezi druhy tak proměnlivá, není zcela objasněn (Lifjeld *et al.* 2019). Mezi hlavní faktory, indikující mezidruhovou míru EPP, patří hnízdní ekologie ve smyslu příležitosti ke spárování, jako je hnízdní denzita a synchronizace (Westneat *et al.* 1990) či párovací systém (Hasselquist & Sherman 2001). Dalšími faktory mohou být různé socio-ekologické aspekty, např. intenzita rodičovské péče ze strany samce (Gowaty 1996), sezónní migrace (Spottiswoode & Møller 2004), hnízdění v tropické versus temperátní zóně (Stutchbury & Morton 2001) nebo pohlavní výběr (Bennett & Owens 2002). Recentní analýza více než dvou set druhů pěvců naznačuje, že promiskuitnější druhy mají (1) sníženou parentální péči, zejména během časné fáze hnízdního cyklu, (2) krátkodobější sociální svazky, (3) vyšší stupeň sexuálního dichromatismu, (4) silnější pohlavní výběr v době před uzavřením sociálního svazku a (5) silnější migrační chování – rezidentní druhy vykazují nejnižší stupeň EPP (Lifjeld *et al.* 2019). Přestože se zdá, že by některé socio-ekologické prediktory mohly vysvětlovat míru samičí promiskuity, nalezené asociace byly spíše slabé (Lifjeld *et al.* 2019). Evoluce chování vedoucího k mimo-párovým paternitám zůstává nepochopena také proto, že obecně předpokládané výhody a nevýhody nevěrného chování pro samice nejsou jednoznačně

dokumentované (Griffith *et al.* 2002; Arnqvist & Kirkpatrick 2005). Vysvětlení samičí promiskuity v kontextu ekologických a evolučních aspektů, včetně pochopení tak markantní variability v míře EPP mezi ptačími druhy, představuje proto stále jednu z hlavních výzev evoluční biologie.

Většina adaptivních vysvětlení samičího promiskuitního chování („adaptivního modely“) je založena na předpokladu výhod plynoucích samicím z mimo-párových kopulací (extra-pair copulation – EPC). V souvislostí s tím bylo navrženo několik hypotéz (Tabulka č. 1). Obecně lze výhody, plynoucí pro samice z promiskuitního chování, rozdělit na přímé, ve smyslu zisku potravního zdroje či teritoria, případně pojištění proti neplodnosti sociálního partnera (Wetton & Parkin 1991; Gray 1997;) a výhody nepřímé, tedy genetické, ze kterých profitují především potomci (Mays & Hill 2004). Nejvíce pozornosti je v empirických studiích upíráno právě na genetické zisky: (1) Hypotéza dobrých genů - samice se mimo svůj vlastní svazek páří s kvalitnějším samcem, než je její sociální partner, čímž získává tzv. „dobré geny“ pro své potomky (Petrie & Kempenaers 1998; Westneat *et al.* 1990; Mays & Hill 2004), (2) Hypotéza komplementárních genů - samice vytváří lepší genetický profil mláďat, ve smyslu vyšší heterozygotnosti, díky výběru partnera s co nejvíce komplementárním genomem ke genomu svému (Kempenaers *et al.* 1999; Tregenza & Wedell 2000; Suter *et al.* 2007; Mays & Hill 2004; Mays *et al.* 2008), (3) Hypotéza genetické diverzity – samice využívá polyandrii ke zvýšení genetické rozmanitosti svých potomků (Williams 1975; Westneat *et al.* 1990).

Navzdory množství hypotéz a studií, snažících se dané predikce potvrdit či vyvrátit, nejsou výhody pro samice zcela jasné a jejich evidence zůstává nejednoznačným a poměrně kontroverzním tématem (Fortsmeier *et al.* 2011; Fortsmeier *et al.* 2014). Samice navíc mohou kopulací s jiným samcem riskovat i určité nevýhody, např. snížení rodičovské péče ze strany sociálního (podváděného) partnera (Dixon *et al.* 1994; Westneat & Sargent 1996; Albrecht *et al.* 2006), zvýšení potenciálního rizika přenosu pohlavně přenosných patogenů a infekcí (Kokko *et al.* 2002a) či zvýšení energetických a časových nákladů při hledání vhodného partnera (Petrie & Kempenaers 1998). To vše vedlo k nutnosti navržení tzv. „neadaptivních modelů“ evoluce samičí promiskuity, které předpokládají, že mimo-párové paternity se mohou vyvinout a v populaci udržet i přesto, že nepřinášejí samicím žádné výhody (Arnqvist & Kirkpatrick 2005; Forstmeier *et al.* 2011; Hsu *et al.* 2015; Lyu *et al.* 2018), a doplňují tak náš pohled na evoluci promiskuitního chování v sociálně monogamních systémech.

Jedním z neadaptivních vysvětlení samičí nevěry může být genetická korelace – samice mohou aktivně vyhledávat EPC v důsledku pleiotropně antagonistického působení genů řídících promiskuitní chování u samců (Fortsmeier *et al.* 2011). Alternativním vysvětlením pro

samičí nevěru mohou být vynucené kopulace ze strany samců, tzv. „hypotéza samčí manipulace“ (Fosrtmeier *et al.* 2014; Hsu *et al.* 2015). Neadaptivní hypotézy jsou však, ve srovnání s hypotézami adaptivními stále poměrně opomíjené, zejména co se týká interpretace nalezených vztahů mezi fenotypovým znakem samce a jeho úspěšností v mimo-párové paternitě (**Publikace 4**). Predikce nejčastěji citovaných hypotéz snažících se vysvětlit výskyt EPP u sociálně monogamních druhů ptáků jsou sumarizovány níže (Tabulka č. 1).

Tabulka č. 1: Hypotézy vysvětlující promiskuitní chování u sociálně monogamních druhů (modifikováno z Hsu *et al.* 2015).

Model	Hypotéza	Popis	Předpoklad
Adaptivní	Dobré geny	Samice se účastní EPC za účelem zisku kvalitních genů pro své potomstvo. Samice jsou schopny rozpoznat kvalitu samce na základě fenotypových znaků, např. kondičně-závislých ornamentů.	Mimo-pároví samci jsou větší, s výraznějšími ornamenty a mohou být starší než sociální samec dané samice.
	Genetická komplementarita	Samice se účastní EPC za účelem maximalizovat genetickou kompatibilitu mezi svým genotypem a genotypem otce svých mláďat. Samice jsou schopny rozpoznat genetickou podobnost na základě fenotypových znaků samce a/nebo skryté samičí volby.	Mimo-pároví samci jsou geneticky odlišnější s danou samicí, ve srovnání s jejím sociálním samcem.
	Genetická diverzita	Samice se účastní EPC za účelem maximalizovat genetickou diverzitu svého potomstva, ale nejsou schopny rozpoznat genetickou podobnost samců.	Mimo-pároví samci se neliší od podváděných samců. Častý je výskyt dvou a více mimo-párových samců v jednom hnízdě s mixovanou paternitou.
Neadaptivní	Genetické omezení	Samice se páří s mimo-párovými samci, ačkoliv z toho pro ně neplyne žádná výhoda, kvůli genetické korelaci mezi pohlavím. Alely, které udržují promiskuitní chování u samců jsou pod silnou pozitivní selekcí, a díky pleiotropnímu efektu zapříčiňují tyto alely promiskuitní chování také u samic.	Mimo-pároví samci se neliší od podváděných samců ani vzhledově, ani kvalitativně.
	Samčí manipulace	Samice se páří s mimo-párovými samci, protože samci je k tomu donutí.	Mimo-pároví samci jsou větší a starší než podvádění samci.

Druhotné pohlavní znaky a jejich signalizace

Druhotné pohlavní znaky jsou často věkově a kondičně závislé, což z nich činí vhodné kandidáty na čestné signály kvality svého nositele, podávající informaci např. o hormonálním, zdravotním či nutričním stavu jedince (Thompson *et al.* 1997; Ohlsson *et al.* 2002; Scheuber *et al.* 2003). Mechanismy, způsobující variabilitu v expresi těchto znaků v závislosti na genetické kvalitě, však nejsou zcela pochopeny.

Exprese sexuálních znaků může být ovlivněna individuálními fixními faktory jako je genotyp jedince, stejně tak jako dynamickými faktory, např. věkem či environmentálně indukovanou genovou expresí (Soulsbury *et al.* 2018). Hovoříme-li o kondičně závislých znacích, je důležité pokusit se nějak definovat samotnou kondici jedince. Tu můžeme rozdělit do několika úrovní (Hill 2011). Somatická část zahrnuje fenotyp, tedy projev znaku navenek, který je ovlivněn také okolními vlivy prostředí, jako je sociální status jedince, velikost jeho teritoria, počet ektoparazitů, oxidativní stres a jiné. Další složku představuje genotyp ve smyslu kvality genetické informace, kterou je jedinec schopen předat svým potomkům (např. heterozygotnost). Poslední komponentou kondice je epigenetická část, tedy mechanismy na úrovni DNA, které nemění sekvenci nukleotidů. Příkladem může být metylace DNA, proces, jenž vede ke snížení genové exprese, případně k úplné inaktivaci genu (Yin *et al.* 2017). Tato dynamická modifikace DNA může mít zásadní vliv na fenotypové znaky (Verhulst *et al.* 2016) a může být kritickou komponentou kondičně závislé exprese ornamentů, např. melaninového zbarvení (Soulsbury *et al.* 2018).

Původně navržená a široce citovaná hypotéza handicapu, vycházející z předpokladu, že ornament musí být pro svého nositele nákladný a v jistém smyslu ho znevýhodňovat, aby mohl čestně reflektovat kondici (Zahavi 1975; Grafen 1990), se zdá být v poslední době překonána (Penn & Számadó 2020; **Publikace 2**). Jako nejpravděpodobnější způsob signalizace kvality jedince se podle posledních dat jeví optimální investice do fenotypu, která je dána vyvážením konkrétní míry exprese a reálnou nákladností na vývoj i udržení daného znaku (Penn & Számadó 2020), tedy investice dle skutečného aktuálního fyziologického stavu jedince (Huttegger *et al.* 2015; Penn & Számadó 2020).

Rozsah signalizační funkce jednotlivých druhotných pohlavních znaků není konstantní. Stejně jako lze nalézt značnou variabilitu v expresi pohlavně selektovaných znaků mezi různými druhy, ale i v rámci jednotlivých populací stejného druhu, mohou se uplatňovat také rozdíly, s jakými ornamenty reflektují kondici a životaschopnost jedince (Møller & Pomiankowski 1993; Iwasa & Pomiankowski 1994; **Publikace 2**). Tato variabilita se navíc

může projevovat v závislosti na konkrétních environmentálních podmínkách či věkových kategoriích studovaných jedinců (Giery & Layman 2015; Romano *et al.* 2017a; **Publikace 3**).

Mimo-párové paternity, pohlavní výběr a samčí fenotyp

Pohlavní výběr neboli sexuální selekce je jednou z forem výběru přirozeného, který je často považován za hlavní hnací sílu v evoluci fenotypu (Darwin 1871). Ve srovnání s výběrem přírodním může ovšem působit protichůdně, neboť exprese znaků, optimalizujících úspěch v párování, je energeticky nákladná (Fisher 1930; Zahavi 1975, ale viz Penn & Számadó 2020) a obvykle neoptimalizuje jiné komponenty fitness, jako je přežívání (Darwin 1871; Andersson 1994). V některých případech mohou ovšem oba typy selekce produkovat stejný směr evoluční změny na fenotyp, a v dané situaci je obtížné rozlišit, který typ výběru je zodpovědný za rozšířený znak/ornament (Simmons & Scheepers 1996).

V případě vícečetných kopulací lze pohlavní výběr rozdělit na dvě časově oddělené části – pre-kopulační a post-kopulační (Birkhead & Pizzari 2002). Na obě fáze je navíc možné nahlížet z pohledu samce (intrasexuální kompetice mezi samci o partnerku a následná možná kompetice spermií v samičím traktu) či z pohledu samice (intersexuální samičí výběr a následná skrytá samičí volba toho, která spermie oplodní vajíčko).

Úspěšnost samce v zisku mimo-párových kopulací je považována za jeden z možných důkazů samičí preference pro určitý samčí znak (Sundberg & Dixon 1996). Ukázkovým příkladem druhotných pohlavních znaků u ptáků jsou péřové ornamenty, které díky své rozmanitosti v intenzitě barvy a velikosti přitahují značnou pozornost ve výzkumu pohlavního výběru. Na výsledné expresi péřových ornamentů se podílejí parametry jako je dostupnost potravních zdrojů (Hill & Montgomerie 1994; McGraw *et al.* 2002), míra parazitace (Hamilton & Zuk 1982) či vnější podmínky v době pelichání (Walker *et al.* 2013).

S objevem mimo-párových paternit bylo opakovaně korelováno mnoho fenotypových znaků samců s jejich úspěchem v EPC, avšak s rozdílnými výsledky. Zatímco některé studie dokumentují pozitivní vztah mezi expresí sekundárních pohlavních znaků samce a počtem zplozených mimo-párových potomků (Saino *et al.* 1997a; Albrecht *et al.* 2009; Thusius *et al.* 2001), v jiných studiích nebyl takový vztah nalezen (Griffith *et al.* 1999; Westneat & Stewart 2003; **Publikace 4**).

Interpretace samičí preference pro více ornamentovaného samce může být ovlivněna několika dalšími, v paternitních studiích často opomíjenými, faktory. Exprese mnoha sexuálně selektovaných znaků roste s věkem (Freeman-Gallant *et al.* 2010, **Publikace 2**), a proto může

být vztah mezi úspěchem samce v EPP a ornamentací ovlivněn efektem samčího stáří, a tedy i určitou zkušeností samce (Freeman-Gallant *et al.* 2010; Lifjeld *et al.* 2011). Mimo-pároví samci jsou často starší a/nebo větší než samci, kteří nezískali žádný úspěch v mimo-párových kopulacích (Cleasby & Nakagawa 2012; Hsu *et al.* 2015; **Publikace 4**). To je poměrně robustní zjištění ve studiích zabývajících se mimo-párovými paternitami u ptáků. Naopak, evidence vyšší exprese ornamentálních znaků u samců úspěšných v EPC je poměrně slabá (Akçay & Roughgarden 2007; Freeman-Gallant *et al.* 2010; Lifjeld *et al.* 2011).

V čem tkví výhoda starších samců nad mladšími, není zcela jasné. Většina studií vidí v nalezeném vztahu podporu pro hypotézu dobrých genů, protože samci, kteří se dožili vyššího věku, prokázali svou životaschopnost a jsou tudíž považováni za geneticky kvalitní jedince (Akçay & Roughgarden 2007; Griffith *et al.* 2002). Věk sám o sobě proto může být samicemi preferovaný znak (Sundberg & Dixon 1996; Tarof *et al.* 2012). Na druhé straně mohou být starší samci zatíženi vyšším množstvím germinálních mutací, čímž se jejich genetická kvalita naopak s rostoucím věkem snižuje (Hansen & Price 1999).

Separovat efekt dobrých genů od výhod spojenými s velikostí a/nebo zkušeností samce je však často obtížné (Alatalo *et al.* 1986). Větší a starší (zkušenější) samci mohou získat reprodukční výhodu díky jejich lepší schopnosti uhlídat svou sociální partnerku a ochránit si tak vlastní paternitu (Møller 1987a) a/nebo lepší schopnosti přimět samici ke kopulaci (Burg & Croxall 2006), jak predikuje neadaptivní model promiskuity (Tabulka č. 1). Alternativně mohou mít starší samci výhodu nad mladšími jedinci v post-kopulační fázi pohlavního výběru, díky vyšší alokaci do kvality ejakulátu (Gonzáles-Solís & Becker 2002; Girndt *et al.* 2018, 2019).

Obecně nabírá pohlavní výběr na síle vždy, když určitý fenotypový znak vede k nenáhodným rozdílům v reprodukčním úspěchu mezi jedinci stejného pohlaví (Arnold & Wade 1984; Andersson 1994). Proto je snazší pochopit sexuální dimorfismus u druhů s polygynním párovacím systémem, kde je vytvářena vysoká variabilita v reprodukční úspěšnosti samců – mnoho samců zůstane nespárovaných, zatímco jiní získají několik partnerek na úkor ostatních (Kirkpatrick 1987). Nicméně, i mnoho sociálně monogamních druhů vykazuje známky silného pohlavního výběru, což může naznačovat existenci skrytých zdrojů reprodukční kompetice mezi samci (Webster *et al.* 2007). Podle Darwina se sexuální selekce v monogamním systému může uplatňovat, pokud v populaci není vyrovnaný poměr pohlaví a někteří samci získají partnerku, zatímco jiní ne, případně pokud určití samci přitahují kvalitnější samice, které jsou schopny zplodit více potomků, čímž získávají reprodukční výhodu nad ostatními samci v populaci (Darwin 1871). Dalším uvažovaným vysvětlením

zmíněného paradoxu jsou právě mimo-párové kopulace, které mohou zásadně ovlivnit sílu sexuální selekce, a to díky možné redistribuci reprodukčního úspěchu mezi samci, kterou v populaci vytvářejí (Arnold & Wade 1984; Whittingham & Dunn 2005; Albrecht *et al.* 2007; Vedder *et al.* 2011; Schlicht & Kempenaers 2013). Tato reprodukční strategie tak může být zdrojem pohlavního výběru zodpovědného za vznik extravagantních ornamentů i u sociálně monogamních druhů (Smith et al 1991; Webster *et al.* 1995; Kempenaers *et al.* 1997; Owens & Hartley 1998). Evidence pro takové tvrzení je však zatím není dostatečně silná (Dunn *et al.* 2001; Freeman-Gallant *et al.* 2005; Akçay & Roughgarden 2007) a otázka, jakou roli mohou mimo-párové paternity sehrát v intenzitě sexuální selekce, čeká na své zodpovězení (Webster *et al.* 1995; Webster *et al.* 2001; Whittingham & Dunn 2005). Daná problematika si proto v budoucnu zaslouží více pozornosti.

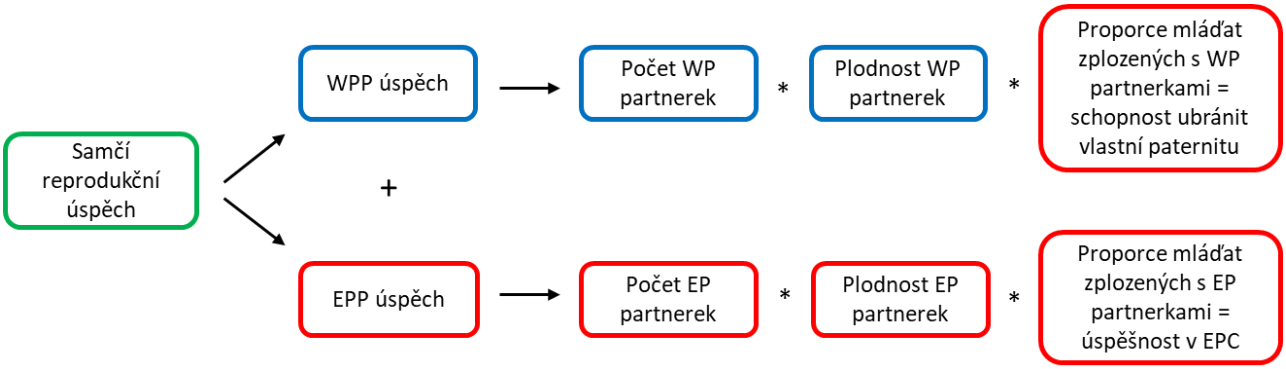
Komponenty reprodukčního úspěchu

Pro správné pochopení promiskuity a evoluce sexuálně selektovaných znaků je nezbytné stanovit jednotlivé komponenty reprodukčního úspěchu samců. Vnitro-párová fertilizace (within-pair fertilization, WPF) a mimo-párová fertilizace (extra-pair fertilization, EPF) jsou dva odlišné způsoby, jak dosáhnout reprodukčního úspěchu, které mohou operovat současně, ale mohou také působit protichůdně (Webster *et al.* 1995). Obě části reprodukčního úspěchu mají několik dalších komponent – počet partnerek, jejich plodnost a proporce zplozených potomků v jejich hnízdě (Obrázek č. 1). Zatímco proporce genetických mláďat v sociálním hnízdě je složkou vnitro-párového úspěchu (WPP úspěch) a indikuje sníženou schopnost samce ochránit vlastní paternitu, proporce mláďat zplozených mimo sociální hnízdo je složkou mimo-párového úspěchu (EPP úspěch), který odráží schopnost samce získat mimo-párovou partnerku.

Mnoho studií, zabývající se pohlavním výběrem, používá k vyjádření párovacího úspěchu samce počet jeho sociálních partnerek nebo počet pozorovaných kopulací, a k vyjádření reprodukčního úspěchu potom počet mláďat v sociálním hnízdě. Nicméně někteří samci mohu přitahovat kvalitnější (plodnější) partnerky (Kirkpatrick *et al.* 1990) a jak samci, tak samice, se mohu účastnit mimo-párových kopulací (Westneat *et al.* 1990, Birkhead & Møller 1992). Stanovení reprodukčního úspěchu je proto podstatně komplikovanější. Jednotlivé komponenty fitness samců lze rekonstruovat pouze za předpokladu přesného stanovení genetických rodičů všech mláďat v populaci.

V **Publikaci 4** jsme separovali tyto dvě části reprodukčního úspěchu a hodnotili efekt fenotypových znaků u vlaštovky obecné na každý z nich zvlášť.

Obrázek č. 1: Komponenty samčího reprodukčního úspěchu – WPP (vnitro-párový) úspěch a EPP (mimo-párový) úspěch; modifikováno z Webster *et al.* 1995. V červeném rámečku jsou vyznačené komponenty, které je možné stanovit až po analýze genetické paternity.



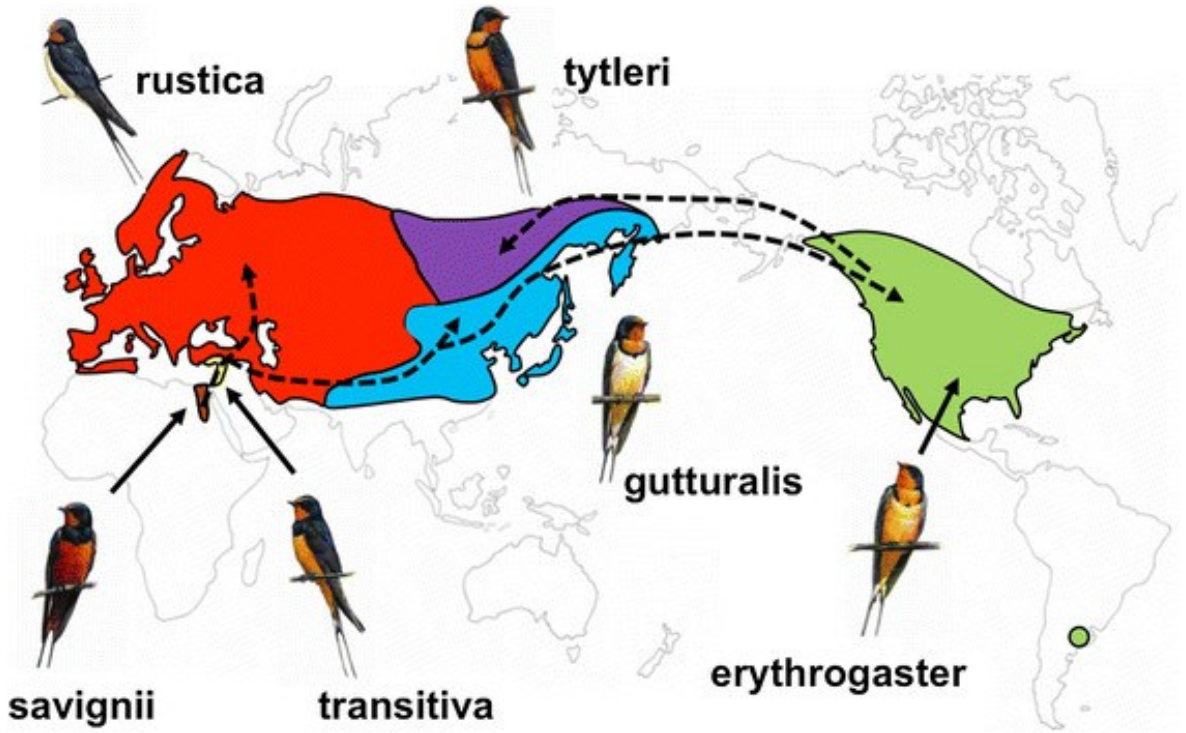
Pohlavní výběr u vlaštovky obecné

(1) Studovaný druh

Vlaštovka obecná (*Hirudno rustica*) je sociálně monogamní pěvec, který se stal kromě symbolu jara také ikonickým objektem studia sexuální selekce. Vidlicovitě vykrojený ocas totiž patří mezi učebnicové příklady pohlavně selektovaných znaků a vlaštovka obecná se tak v poslední době řadí mezi modelový druh právě ve výzkumu pohlavního výběru a mimo-párových paternit (Møller 1994a; Møller *et al.* 1998; Romano *et al.* 2017a).

Druh *Hirundo rustica*, skládající se z šesti příbuzných poddruhů, vykazuje téměř celosvětové rozšíření a fenotypovou variabilitu zejména v délce ocasních per a intenzitě melaninového zbarvení hrdelní a ventrální části těla – Obrázek č. 2 (Dor *et al.* 2010; Scordato & Safran 2014; Safran *et al.* 2016). Na konci rýdovacích per se nacházejí bílé skvrny bez pigmentu, jejichž plocha pozitivně koreluje s délkou rýdovacích per, a které se mohou uplatňovat nejen ve vnitrodruhové vizuální komunikaci (Møller 1994a), ale také v pohlavním výběru (Kose & Møller 1999; Saino *et al.* 2015). Právě tyto tři znaky se staly objektem rozsáhlého studia pohlavního výběru vlaštovek.

Obrázek č. 2: Mapa rozšíření šesti známých podruhů *Hirundo rustica.* Šipky nazančují hypotetický směr kolonizace od společného afrického předka. Obrázek převzat z práce Scordato & Safran 2014.



Mezi nejvíce prostudované poddruhy patří *H. r. rustica* s výskytem od západní Evropy po východní Rusko a severní Afriku a *H. r. erythrogaster* vyskytující se převážně v Severní Americe. Tyto dva poddruhy se liší v expresi fenotypových znaků – zatímco evropská vlaštovka má výrazně prodloužená ocasní pera a spíše světlé zbarvení břišní strany těla, severoamerický poddruh má ocasní pera kratší a ventrální část těla rezavě zbarvenou (Møller 1994a; Safran *et al.* 2005; Romano *et al.* 2017a).

Fenotypová rozdílnost mezi jednotlivými poddruhy je spojována s jejich divergencí (Vortman *et al.* 2013; Scordato & Safran 2014; Safran *et al.* 2016), která není dosud kompletní, jak naznačuje hybridizace a tok genů vyskytující se na většině hranic poddruhů (Dor *et al.* 2012; Wilkins *et al*. 2016). Variabilita v expresi ornamentálních znaků existuje také v rámci jednoho poddruhu, v závislosti na geografii, pohlaví či věku jedince, což může vytvářet různou intenzitu sexuální selekce pro daný znak v různých podmínkách (Romano *et al.* 2017a; **Publikace 3**). Geografická variabilita v objektech zájmu samičí volby ve výběru partnera upozorňuje na roli, jakou může pohlavní výběr sehrát v populační divergenci a speciaci u studovaného komplexu vlaštovek (Scordato & Safran 2014).

Párovacím systémem u vlaštovky obecné je sociální monogamie s častým výskytem mimo-párových paternit (Laskemoen *et al.* 2013, **Publikace 4**) a méně častěji dokumentovaným výskytem hnízdního parazitismu a quasi-parazitismu (Møller 1987b; Møller *et al.* 2003, **Publikace 1**). V námi studované populaci evropského poddruhu vlaštovky obecné byl hnízdní parazitismus detekován ve 22 % hnízd, což indikuje mírně vyšší míru parazitace, než bylo dokumentováno v dánské populaci (16.5 %; Møller 1987b). To může být zapříčiněno jiným metodologickým přístupem detekce parazitické události (**Publikace 1**). Zhruba ve 28 % všech parazitovaných hnízd byl odhalen výskyt quasi-parazitismu. Hnízda quasi-parazitických samic nebyla nikdy v těsné blízkosti hnízda hostitelského, což nasvědčuje nenáhodné distribuce této reprodukční strategie. Quasi-parazitismus byl u vlaštovky obecné detekován již dříve, avšak překvapivě v populaci, ve které nebyl popsán výskyt hnízdního parazitismu (Møller 2003). Mimo-párové paternity byly nalezeny v 51 % hnízd sledované populace. Téměř 20 % mláďat bylo zplozeno mimo sociální svazek (**Publikace 4**). Podobná míra EPP byla popsána i v jiných populacích vlaštovky obecné (Saino *et al.* 1999; Møller *et al.* 2003; Kleven *et al.* 2006; Neuman *et al.* 2007; Vortman *et al.* 2011; Lakemoen *et al.* 2013).

Jedinci vlaštovky obecné hnízdí převážně v koloniích, přičemž některé páry stavějí hnízda solitérně, ne v těsném kontaktu s jinými hnízdy (Møller 1987b; Fujita & Higuchi 2007). U těchto párů byla dokumentována menší pravděpodobnost výskytu CBP (Møller 1987b;

Publikace 1), což může ze solitérnosti v hnízdění činit obrannou strategii proti hnízdnímu parazitismu.

(2) Pohlavní výběr

Dospělci vykazují střední stupeň pohlavního dimorfismu v několika znacích, které hrají různou roli v pohlavním výběru v závislosti na konkrétním poddruhu nebo dokonce populaci (Scordato & Safran 2014; Romano *et al.* 2017a; **Publikace 3**). Zatímco u evropského poddruhu se za hlavní ornament pod sexuální selekcí, který zvýhodňuje svého nositele jak ve vnitro-párovém, tak v mimo-párovém úspěchu, považují prodloužená rýdovací pera, případně s nimi korelovaná velikost a tvar bílých ocasních skvrn (Møller 1988a, 1994a, Møller & Tegelström 1997; Saino *et al.* 1997a; ale **Publikace 4**), u severoamerického poddruhu se hlavním znakem, preferovaným samicemi při volbě partnera, zdá být melaninové ventrální zbarvení dosahující různé intenzity (Safran & McGraw 2004; Safran *et al.* 2005, Eikenaar *et al.* 2011, ale Kleven *et al.* 2006). Ke stanovení důležitosti role, jakou hrají tyto ornamenty v samičím výběru partnera, byly u vlaštovky obecné provedeny jak korelativní studie, tak experimentální manipulace obou znaků (Møller 1988a; Smith & Montgomerie 1991; Smith *et al.* 1991; Møller 1994a; Saino *et al.* 1997a; Safran & McGraw 2004; Safran *et al.* 2005; Vortman *et al.* 2013; Safran *et al.* 2016; Romano *et al.* 2017a).

Vzhledem k tomu, že je exprese pohlavních znaků často asociována s věkem svého nositele, mohly by výše zmíněné ornamenty u vlaštovky obecné sloužit jako indikátory životaschopnosti jedince (Jennions *et al.* 2001; **Publikace 2**). Pokud jsou samci s delšími pery atraktivnější pro samice, je možné, že se prodloužená ocasní pera u vlaštovky vyvinula působením sexuální selekce (Møller 1988a, 1994a). Na druhou stranu může délka rýdovacích per představovat od určité hranice náklady na životaschopnost svého nositele (Møller & de Lope 1994). Některé studie předpokládají, že vidlicovitě vykrojený ocas se mohl vyvinout také díky přírodnímu výběru, poněvadž takový tvar zlepšuje letecké schopnosti (Norberg 1994; Buchanan & Evans 2000; Hasegawa *et al.* 2016). V daném případě mluvíme o „hypotéze aerodynamické výhody" – vidlicovité vykrojení ocasu může do určité míry zdokonalovat schopnost manévrování, a to jak v přirozeném prostředí, tak v experimentálně vytvořených podmínkách (Evans 1998; Buchanan & Evans 2000; Park *et al.* 2000). Jaký typ přirozeného výběru je zodpovědný za prodloužená ocasní pera u vlaštovky obecné, není doposud zcela objasněné a obě možnosti jsou často diskutovány a propojovány (Rowe *et al.* 2001; Bro-Jørgensen *et al.* 2007; Aparicio & Møller 2012; Hasegawa & Arai 2020). Pro pochopení je nutné stanovit a testovat nevýhody pro nositele více exprimovaného znaku ve smyslu přežívání a životaschopnosti.

(3) Signalizační funkce rýdovacích per

Jak bylo již zmíněno, ornamenty mohou v rámci druhu zastávat funkci indikátoru kvality jedince (Jennions *et al.* 2001; **Publikace 2**). Náklady na pohlavně selektované znaky stojí proto ve středu pozornosti teorie pohlavního výběru již řadu let. Více ornamentovaní jedinci mohou být znevýhodněni ve smyslu přežívání, neboť mohou být kvůli vyšší atraktivitě vystaveni zvýšenému riziku ze strany predátorů či parazitů a v neposlední řadě musí investovat nemalé energetické náklady na produkci a udržení daného ornamentu (Kotiaho 2001). V takovém případě by se dal očekávat negativní vztah mezi expresí ornamentu a přežíváním (Balbontín *et al.* 2011). Na druhou stranu, u druhů, u kterých by měly pohlavní znaky signalizovat kvalitu a kondici svého nositele, je predikovaná pozitivní závislost intenzity exprese takového znaku s životaschopností jedince (Møller 1991; Jennions *et al.* 2001).

Pokud existuje aktivní preference pro stáří mimo-párového partnera a/nebo partnerky, nabízí se otázka, jakým způsobem mohou jedinci věk svého partnera odhadnout. Exprese ornamentů obvykle roste s věkem (Freeman-Gallant *et al.* 2010), a rýdovací pera u vlaštovky obecné nejsou výjimkou (Lifjeld *et al.* 2011; Balbontín *et al.* 2011). V **Publikaci 2** jsme analyzovali změny v délce ocasních per v závislosti na věku a testovali tak možnost signalizační funkce tohoto ornamentu u dvou geograficky oddělených populací vlaštovky obecné (Česká republika, Rumunsko), a to ve smyslu výpovědní hodnoty o stáří a životaschopnosti jedince. Změny ve fenotypu v závislosti na stáří byly sledovány separátně jak v rámci jednoho jedince, tak mezi jedinci, čímž jsme získali informaci o míře mortality spojené s délkou rýdovacích per. Délka ocasních per rostla s věkem u obou pohlaví, přičemž k nejvýraznější změně docházelo v mladém věku. Nenalezli jsme však žádnou evidenci pro senescenci, jak bylo dokumentováno v jiných evropských populacích (Balbontín *et al.* 2011). Naše výsledky naznačují, že zahrnutí věku coby prediktoru délky ocasních per pouze ve formě polynomu druhého řádu do modelu, lze chybně interpretovat jako senescenci (Bouwhuis *et al.* 2009). Nesoulad mezi našimi výsledky a předchozí studií proto může být způsoben odlišným statistickým přístupem, ale také odlišnou signalizační funkcí studovaného ornamentu v různých populacích (**Publikace 2**).

K otestování potenciálních nákladů spojených s délkou rýdovacích per jsme využili také manipulativního experimentu. V kontrastu s jinými studiemi, ve kterých byla iniciální délka ocasních per spojena s vyšším dožitím svého nositele (Møller & de Lope 1994; Saino *et al.* 1997b), neovlivnila v naší populaci manipulace s délkou rýdovacích per přežívání jedince do další sezóny, ani délku jeho ocasních per v následujícím roce (**Publikace 2**). Nezaznamenali jsme ani odlišný efekt manipulace mezi samci s přirozeně krátkými a dlouhými rýdovacími pery. Výsledky tedy naznačují, že náklady spojené s délkou ocasu jsou v námi studované

populaci spíše nízké a mohou být významné pouze u samců s extrémně prodlouženými pery. Obecně se lze domnívat, že hypotéza handicapu nebude zřejmě hlavním mechanismem uplatňujícím se při pohlavním výběru ve sledované populaci vlaštovek. Je však potřeba vzít v úvahu, že experiment byl proveden mezi prvním a druhým hnízdním pokusem, oproti předchozím studiím, které manipulovaly před začátkem první snůšky (Møller & de Lope 1994; Saino *et al.* 1997b), tedy v nejvíce kritickém období, během nějž se formují páry, které následně staví hnízdo a oba dospělci se snaží odchovat svou první snůšku. Efekt přežívání v závislosti na manipulativním experimentu proto může být v naší studii slabší také z důvodu kratšího a méně intenzivního vystavení vlivu manipulace.

Délka ocasu byla pozitivně asociována s přežíváním a celkovou délkou života, ale pouze u samců, nikoliv u samic. Tento výsledek podporuje signalizační funkci ornamentu ve smyslu životaschopnosti samců. Pozitivní korelace exprese tohoto ornamentu a délky života byla dokumentována také v několika dalších populacích vlaštovky obecné (Møller 1991, 1994b; Saino *et al.* 1997b), zatímco v jiných populacích nabýval zkoumaný vztah negativního trendu (Balbontín *et al.* 2011; Romano *et al.* 2017b). Takovýto rozpor ve výsledcích může signalizovat odlišný mezi-populační vztah mezi expresí ornamentu a přežíváním, což může vést k odlišné síle pohlavního výběru pro daný znak v různých podmínkách (Kokko *et al.* 2002b), např. vlivem různých migračních tras nebo zimovišť (Ambrosini *et al.* 2009; Klvaňa *et al.* 2018).

(4) Reprodukční úspěšnost

Na rozdíl od ostatních studií jsme v české populaci nenalezli dostatečnou podporu pro domněnku, že délka ocasních per je u vlaštovky obecné důležitým prediktorem fertilizačního úspěchu (**Publikace 4**). Ani úspěšnost ve vnitro-párové paternitě, ani úspěch v mimo-párových kopulacích nebyl ovlivněn délkou ocasních per nebo ventrálním zbarvením těla. V párovém srovnání se podvádění samci nelišili ani v jednom ze sledovaných ornamentálních znaků od samců mimo-párových, tedy těch, se kterými je jejich sociální partnerka podvedla. Lišili se pouze v délce tarsu, který je u pěvců považován za ukazatele strukturální velikosti těla (Kempenaers *et al.* 1997; Kruuk *et al.* 2001). Podvádějící samci měli delší tarsus ve srovnání se samci, kteří jimi byli podvedeni, což lze interpretovat jako podporu pro neadaptivní model samičí promiskuity – větší samci mohou být zdatnější ve vynucování kopulací („samčí manipulace“, Hsu *et al.* 2015).

Nejdůležitějším znakem, který hraje roli v reprodukčním úspěchu samce v námi studované populaci, se zdá být stáří jedinců. Schopnost samce získat mimo-párovou partnerku rostl

lineárně s věkem samce, což bylo dokumentováno také u amerického poddruhu (Lifjeld *et al.* 2011). Naše výsledky jsou v souladu s meta-analýzou založenou na více druzích a jejich fenotypových znacích, která naznačuje, že věk je u ptáků obecně důležitějším ukazatelem úspěšnosti v mimo-párové fertilizaci než ornamenty (Hsu *et al.* 2015). Podle hypotézy samčí manipulace mohou být starší samci zkušenější ve vynucování mimo-párových kopulací, a tím být úspěšnější v zisku mimo-párové partnerky (Weatherhead & Boag 1995; Westneat & Stewart 2003; Hsu *et al.* 2015). Naproti tomu jediným prediktorem toho, zda bude samec podveden svou sociální partnerkou, byl věk samice. Starší samice se častěji účastnily mimo-párových kopulací. Interpretace nalezených vztahů závisí na celkovém pohledu na EPP a je v rámci vedené diskuse o evoluci EPP celkem symptomatická. Pokud nahlížíme na promiskuitu jako na samičí strategii za účelem obstarání lepších podmínek či genetických zisků pro své potomky, můžeme častější přítomnost EPP v hnízdech starších samic považovat za důsledek lepší schopnosti vyhnout se hlídání ze strany sociálního partnera u starších, zkušenějších samic (Dietrich *et al.* 2004; Bouwman & Komdeur 2005). Alternativním a z hlediska samic neadaptivním vysvětlením může být teorie, že starší samice jsou preferovány mimo-párovými samci, neboť jsou pro ně atraktivnější ve smyslu fenotypu či kondice. U mnoha ptačích druhů bylo totiž dokumentováno, že starší samice hnízdí dřív, mají větší snůšky a mohou produkovat kvalitnější potomstvo (Cichon *et al.* 2003; Turner 2006; Decker *et al.* 2012). Pokud je plodnost mimo-párových partnerek důležitou složkou samčího reprodukčního úspěchu (Webster *et al.* 1995), pak mohou být starší samice preferenčně vybírány jako mimo-párové partnerky.

Nenalezení vztahu mezi samčím úspěchem (WPP ani EPP) a délkou rýdovacích per v námi studované populaci (**Publikace 4**) může také naznačovat mezi-populační rozdíly v pohlavním výběru nebo v signalizační funkci daného ornamentu (Kokko *et al.* 2002b, **Publikace 3**). Takové rozdíly byly zaznamenány u severoamerického poddruhu vlaštovky (Safran *et al.* 2005; Kleven *et al.* 2006), i u jiných druhů pěvců. Zatímco v dánské populaci vrabce domácího (*Passer domesticus*) byla dokumentována samičí preference pro velikost černé hrdelní skvrny (Møller 1987c, 1988b, 1989), ve španělské populaci nebyl takový vztah nalezen (Veiga 1993; Cordero *et al.* 1999) a v populaci britské byl dokonce dokumentován vztah negativní (Griffith *et al.* 1999). Podobně byly zjištěny rozdíly v sexuálně selektovaných znacích u lejska černohlavého (*Ficedula hypoleuca*; Dale *et al.* 1999; Saetre *et al.* 1995). Geografická variabilita v sexuální selekci na určitý znak proto může vysvětlovat nejen odlišné znaky asociované se samčí atraktivitou v různých populacích, ale také různou sílu, se kterou pohlavní výběr operuje (Scordato & Safran 2014; Romano *et al.* 2017a; **Publikace 3**). Recentní meta-analýza, zabývající se již zmiňovanou velikostí hrdelní skvrny u vrabce domácího, navíc poukazuje

na možnost publikačního zkreslení, které může zásadně změnit platnost dostupné podpory pro jednotlivé hypotézy pohlavního výběru (Sánchez-Tójar *et al.* 2018).

Ačkoliv nebyl v námi studované populaci nalezen vztah mezi délkou ocasních per a mimopárovým úspěchem samce, může se tento ornament, vzhledem k jeho prokázané signalizační funkci, uplatňovat v sociálním párování. Bez otestování samičí preference pro výběr sociálního partnera (např. s využitím manipulativního experimentu před vytvořením sociálních párů, tedy před začátkem prvního hnízdění) a bez detailního zhodnocení jednotlivých komponent reprodukčního úspěchu a vztahu mezi nimi, nejsme schopni plně porozumět funkci tohoto ikonického ornamentu v pohlavním výběru vlaštovek. Další kroky, které by nám pomohly pochopit reprodukční biologii vlaštovky obecné v širším behaviorálně-ekologickém kontextu, by se proto měly ubírat tímto směrem.

CÍLE PRÁCE

- Popsat frekvenci CBP a QP u vlaštovky obecné s využitím molekulárního určení rodičovství, otestovat možné aspekty ovlivňující výskyt těchto strategií v populaci – načasování hnízdění, hnízdní synchronizace, solitérnost x kolonialita (**Publikace 1**).

- Otestovat možnost signalizační funkce rýdovacích per ve smyslu životaschopnosti a stáří jedince u dvou evropských populací vlaštovky obecné (Česká republika, Rumunsko); s využitím manipulativního experimentu otestovat náklady spojené s délkou rýdovacích per u české populace vlaštovky obecné (**Publikace 2)**.

- Kvantifikovat sexuální selekci pro dva potenciální pohlavně selektované znaky – délku rýdovacích per a ventrální zbarvení u dvou populací vlaštovky obecné (Česká republika, Rumunsko) zkoumaných po dobu několika let (**Publikace 3**).

- Určit fenotypové znaky (délka ocasních per, ventrální zbarvení, bílé ocasní skvrny, velikost těla, samčí a samičí věk) asociované s vnitro-párovým a mimo-párovým úspěchem samce vlaštovky obecné (**Publikace 4**).

SHRNUTÍ HLAVNÍCH VÝSLEDKŮ

- Vnitrodruhový hnízdní parazitismus byl detekován ve 22 % hnízd vlaštovky obecné (*Hirundo r. rustica*). Míra hnízdního parazitismu v námi studované české populaci pozitivně korelovala s hnízdní synchronizací samic. Samice, které využily tuto reprodukční strategii v kombinaci s normálním hnízděním, měly v průměru o jedno mládě více než ostatní samice v populaci.
- Zhruba ve 28 % všech parazitovaných hnízd byl dokumentován quasi-parazitismus (parazitické vejce bylo oplodněno rezidentním samcem hnízda). Hnízda quasi-parazitických samic nebyla nikdy umístěna v těsné blízkosti hnízda hostitelského, což naznačuje nenáhodnou distribuci QP.
- Jak CBP, tak QP měl negativní dopad na reprodukční úspěch hostitelské samice. Naopak samci, účastnící se QP, měli reprodukční výhodu ve srovnání s ostatními samci v populaci, což lze pochopit tak, že kooperace s parazitickou samicí může být pro samce výhodná.
- U dvou evropských populací vlaštovky obecné (ČR, Rumunsko) byla délka rýdovacích per asociována s věkem jedince. Nejvyšší nárůst byl zaznamenán mezi prvním a druhým rokem života.
- Samci s delšími rýdovacími pery vykazovali lepší přežívání, což podporuje význam tohoto ornamentu v signální funkci životaschopnosti jedince.
- Experimentální manipulace s délkou rýdovacích per neměla žádný efekt na přežívání, ani na délku ocasních per v následujícím roce, což indikuje nízkou nákladnost tohoto ornamentu. Pro délku rýdovacích per nebyla nalezena evidence stárnutí.
- Exprese dvou vybraných fenotypových znaků (délka rýdovacích per a ventrální zbarvení těla) se lišila mezi jednotlivými studovanými populacemi (ČR, Rumunsko). Česká populace měla signifikantně delší ocasní pera, naopak jedinci z rumunské populace vykazovali tmavší melaninové zbarvení.
- Byly zaznamenány rozdílnosti v obou sledovaných znacích mezi pohlavím a věkovými třídami v rámci jedné populace. Selekce pro délku rýdovacích per byla silnější u mladších (poprvé hnízdících) jedinců a u samců v rumunské populaci, zatímco v české populaci nebyly takové rozdíly detekovány. Tmavší zbarvení se častěji vykytovalo u samců a starších jedinců, což by mohlo signalizovat význam tohoto ornamentu v pohlavním výběru evropského poddruhu vlaštovky obecné.

- Mimo-párové paternity byly detekovány v 51 % hnízd sledované populace. Z celkového počtu 694 mláďat bylo téměř 20 % zplozeno mimo sociální svazek.
- V kontrastu s předchozími studiemi na evropském poddruhu vlaštovky obecné nebyl v námi studované české populaci nalezen průkazný vztah mezi délkou rýdovacích per samce a jeho úspěšností v paternitě (ani mimo-párové, ani vnitro-párové). Hlavním prediktorem obou komponent reprodukčního úspěchu se zdá být stáří jedince (starší samci byli úspěšnější v zisku mimo-párové partnerky, naopak samci spárovaní se starší samicí častěji ztráceli paternitu ve vlastním hnízdě).
- Podvádění a podvádějící samci se v párovém srovnání nelišili v žádném ze sledovaných ornamentálních znaků, pouze ve strukturální velikosti vyjádřené délkou tarsu (mimo-pároví samci měli tarsus delší než samci, kteří jimi byli podvedeni).

ZÁVĚR

V předložené práci jsme se zabývali třemi alternativními reprodukčními strategiemi v populaci vlaštovky obecné (*Hirundo rustica rustica*). Ačkoliv je vlaštovka modelovým druhem v oblasti behaviorální ekologie a mnoho studií se v posledních třech dekádách zaměřilo na genetické určení rodičovství, vnitrodruhový hnízdní parazitismus nebyl u tohoto druhu dosud detekován s využitím molekulárních metod. Parazitismus jsme pozorovali téměř u jedné čtvrtiny hnízd, přičemž 28 % všech parazitických mláďat bylo zplozeno sociálním samcem parazitované samice. Hnízda quasi-parazitických samic nebyla nikdy v přímé blízkosti hostitelského hnízda a zdá se, že samci mohou pomocí této alternativní taktiky zvyšovat svůj reprodukční úspěch (**Publikace 1**).

U dvou evropských populací vlaštovky obecné (české a rumunské) jsme testovali možnost, zda délka ocasních per dokáže odrážet individuální věk a životaschopnost jedince ve smyslu přežívání (**Publikace 2**). Naše výsledky podporují obě hypotézy – prodloužená rýdovací pera signalizují jak stáří jedince, tak jeho viabilitu, ačkoliv i zde byly nalezeny mezi-populační rozdíly. Délka ocasních per se u samců i samic prodlužuje s věkem, funkci kvality navíc podporuje lepší přežívání samců s delším ocasem.

Pro délku rýdovacích per jsme u obou studovaných populací prokázali signifikantní usměrňující selekci, podobně jako jiné studie (Costanzo *et al.* 2017). Rozsah selekce se vak lišil nejen mezi populacemi, ale také mezi pohlavím a věkovými skupinami. To indikuje, že vliv selekce se může s věkem měnit a může se lišit dokonce i mezi blízce příbuznými populacemi (**Publikace 3**).

Oproti ostatním pracím jsme v námi studované české populaci nenalezli podporu pro hypotézu, že délka rýdovacích per a melaninové ventrální zbarvení hraje zásadní roli v samčí reprodukční úspěšnosti. Hlavním prediktorem úspěchu v mimo-párové a vnitro-párové paternitě byl věk samce, respektive samice (**Publikace 4**). V párovém srovnání jsme nenalezli žádný rozdíl ve sledovaných fenotypových znacích mezi podváděným a podvádějícím samcem. Naše výsledky jsou v souladu s meta-analýzou, založené na různých pohlavně selektovaných znacích u různých ptačích druhů, která naznačuje, že nejdůležitějším znakem uplatňujícím se v mimo-párových paternitách je věk samce, nikoliv jeho ornamenty (Hsu *et al.* 2015). Ornament se však může uplatňovat ve výběru sociálního partnera. K objasnění obecně přijímaného paradigmatu, že délka ocasních per u vlaštovky je klíčovým znakem ve volbě partnera, jsou potřebné další informace z různých populací tohoto druhu.

REFERENCE

Akçay, E., & Roughgarden, J. (2007). Extra-Pair Paternity in Birds: Review of the Genetic Benefits. *Evolutionary Ecology Research, 9* (5), 855–868.

Alatalo, R. V., Lundberg, A., & Glynn, C. (1986). Female pied flycatchers choose territory quality and not male characteristics. *Nature*, *323* (6084), 152–153.

Albrecht, T., Kreisinger, J., & Pialek, J. (2006). The strength of direct selection against female promiscuity is associated with rates of extrapair fertilizations in socially monogamous songbirds. *American Naturalist, 167*, 739-744.

Albrecht, T., Schnitzer, J., Kreisinger, J., Exnerova, A., Bryja, J., & Munclinger, P. (2007). Extrapair paternity and the opportunity for sexual selection in long-distant migratory passerines. *Behavioral Ecology*, *18* (2), 477–486.

Albrecht, T., Vinkler, M., Schnitzer, J., Poláková, R., Munclinger, P., & Bryja, J. (2009). Extra-pair fertilizations contribute to selection on secondary male ornamentation in a socially monogamous passerine. *Journal of Evolutionary Biology*, *22* (10), 2020–2030.

Alves, M. A. S., & Bryant, D. M. (1998). Brood parasitism in the sand martin *Riparia riparia*: Evidence for two parasitic strategies in a colonial passerine. *Animal Behaviour*, *56* (6), 1323–1331.

Ambrosini, R., Møller, A.P. & Saino, N. (2009). A quantitative measure of migratory connectivity. *Journal of Theoretical Biology*, *257*, 203–211.

Andersson, M. (1984). *Brood parasitism within species*. Pp. 195-227 in Producers and scroungers: strategies of exploitation and parasitism (C. J. Barnard, Ed.). London, Croom Helm.

Andersson, M. (1994). *Sexual selection*. Princeton University Press, Princeton, US-NJ.

Andersson, M., & Åhlund, M. (2000). Host-parasite relatedness shown by protein fingerprinting in a brood parasitic bird. *Proceedings of the National Academy of Sciences of the United States of America*, *97*, 13188–13193.

Andersson, M., & Åhlund, M. (2001). Protein fingerprinting: a new technique reveals extensive conspecific brood parasitism. *Ecology*, *82*, 1433–1442.

Andersson, M., & Åhlund, M. (2012). Don't put all your eggs in one nest: spread them and cut time at risk. *The American Naturalist 180* (3), 354–363.

Andersson, M., Åhlund, M., & Waldeck, P. (2019). Brood parasitism, relatedness and sociality: A kinship role in female reproductive tactics. *Biological Reviews of the Cambridge Philosophical Society*, *94,* 307-327.

Aparicio, J., & Møller, A. (2012). Artefactual effects of tail manipulation on fitness. *Animal Behaviour*, *83*, e1–e3.

Arnold, S., & Wade, M. (1984). On the Measurement of Natural and Sexual Selection: Applications. *Evolution*, *38*, 720–734.

Arnold, K. E., & Owens, P. F. (2002). Extra-pair paternity and egg dumpling in birds: life history, parental care and the risk of relation. *Proceedings of the Royal Society B: Biological Sciences, 269,* 1263-1269.

Arnqvist, G., & Kirkpatrick, M. (2005). The Evolution of Infidelity in Socially Monogamous Passerines: The Strength of Direct and Indirect Selection on Extrapair Copulation Behavior in Females. *The American Naturalist*, *165,* 26-37.

Balbontín, J., de Lope, F., Hermosell, I., Mousseau, T., & Møller, A. (2011). Determinants of age-dependent change in a secondary sexual character. *Journal of evolutionary biology*, *24*, 440–448.

Bennett, P. M., & Owens, I. P. F. (2002). Evolutionary ecology of birds: life histories, mating systems and extinction. Oxford, Oxford University Press.

Berger, I., Dvir, Y., Leshem, Y., Yom-Tov, Y., & Markman, S. (2014). Extra-pair copulations, intra-specific brood parasitism, and quasi-parasitism in birds: A theoretical approach. *Acta Ethologica*, *17*, 131-140.

Birkhead, T. R., & Møller, A. P. (1992). *Sperm competition in birds: evolutionary causes and consequences.* Academic Press, London.

Birkhead, T. R., & Pizzari, T. (2002). Postcopulatory sexual selection. *Nature Reviews Genetics, 3*, 262-273.

Bouwhuis, S., Sheldon, B.C., Verhulst, S., & Charmantier, A. (2009). Great tits growing old: selective disappearance and the partitioning of senescence to stages within the breeding cycle. *Proceedings of the Royal Society B: Biological Sciences, 276*, 2769–2777.

Bouwman, K. M., & Komdeur, J. (2005). Old Female Reed Buntings (*Emberiza schoeniclus*) Increase Extra-Pair Paternity in Their Broods When Mated to Young Males. *Behaviour*, *142*, 1449–1463.

Bro-Jørgensen, J., Johnstone, R. A., & Evans, M. R. (2007). Uninformative Exaggeration of Male Sexual Ornaments in Barn Swallows. *Current Biology*, *17* (10), 850–855.

Brouwer, L., & Griffith, S. C. (2019). Extra-pair paternity in birds. *Molecular Ecology*, *28* (22), 4864–4882.

Brown, C. R., & Brown, M. B. (1991). Selection of high-quality host nests by parasitic cliff swallows. *Animal Behaviour*, *41* (3), 457–465.

Buchanan, K. L., & Evans, M. R. (2000). The effect of tail streamer length on aerodynamic performance in the barn swallow. *Behavioral Ecology*, *11* (2), 228–238.

Burg, T. M., & P. Croxall, J. (2006). Extrapair paternities in black-browed *Thalassarche melanophris*, grey-headed *T. chrysostoma* and wandering albatrosses *Diomedea exulans* at South Georgia. *Journal of Avian Biology*, *37* (4), 331–338.

Cichon, M. (2003). Does prior breeding experience improve reproductive success in Collared Flycatcher females? *Oecologia*, *134*, 78–81.

Cleasby, I. R., & Nakagawa, S. (2012). The influence of male age on within-pair and extra-pair paternity

in passerines. *Ibis*, *154* (2), 318–324.

Clutton-Brock, T. H. (1991). *The evolution of parental care*. Princeton University Press.

Cordero, P. J., Wetton, J. H., & Parkin, D. T. (1999). Extra-Pair Paternity and Male Badge Size in the House Sparrow. *Journal of Avian Biology*, *30* (1), 97–102.

Costanzo, A., Ambrosini, R., Caprioli, M., Gatti, E., Parolini, M., Canova, L., Rubolini, D., Romano, A., Gianfranceschi, L., & Saino, N. (2017). Lifetime reproductive success, selection on lifespan, and multiple sexual ornaments in male European barn swallows. *Evolution, 71*, 2457-2468.

Dale, S., Slagsvold, T., Lampe, H. M., & Sætre, G.-P. (1999). Population Divergence in Sexual Ornaments: The White Forehead Patch of Norwegian Pied Flycatchers Is Small and Unsexy. *Evolution*, *53* (4), 1235–1246.

Darwin, C. R. (1871). *The Descent of Man and Selection in Relation to Sex*. John Murray, London, UK.

Davies, N. B. (2000). Cuckoos, Cowbirds and Other Cheats. *London, Poyser.*

Dawkins, R. (1980). Good strategy or evolutionarily stable strategy. In G. W. Barlow & J. Silverberg (Eds.), *Sociobiology: Beyond nature/nurture* (pp. 331–367). Boulder: Westview Press.

Decker, K., Conway, C., & Fontaine, J. (2012). Nest predation, food, and female age explain seasonal declines in clutch size. *Evolutionary Ecology*, *26*, 683–699.

Dietrich, V., Schmoll, T., Winkel, W., Epplen, J. T., & Lubjuhn, T. (2004). Pair Identity: An Important Factor concerning Variation in Extra-Pair Paternity in the Coal Tit (*Parus ater*). *Behaviour*, *141* (7), 817–835.

Dixon, A., Ross, D., O'Malley, S. L. C., & Burke, T. (1994). Paternal investment inversely related to degree of extra-pair paternity in the reed bunting. *Nature*, *371* (6499), 698–700.

Dor, R., Safran, R. J., Sheldon, F. H., Winkler, D. W., & Lovette, I. J. (2010). Phylogeny of the genus Hirundo and the Barn Swallow subspecies complex. *Molecular Phylogenetics and Evolution*, *56* (1), 409–418.

Dor, R., Safran, R. J., Vortman, Y., Lotem, A., McGowan, A., Evans, M. R., & Lovette, I. J. (2012). Population genetics and morphological comparisons of migratory European (*Hirundo rustica rustica*) and sedentary East-Mediterranean (*Hirundo rustica transitiva*) barn swallows. *The Journal of Heredity*, *103* (1), 55–63.

Dunn, P. O., Whittingham, L. A., & Pitcher, T. E. (2001). Mating Systems, Sperm Competition, and the Evolution of Sexual Dimorphism in Birds. *Evolution*, *55* (1), 161–175.

Eadie, J. M. (1989). Alternative reproductive tactics in a precocial bird: The ecology and evolution of brood parasitism in goldeneyes. PhD Thesis, University of British Columbia.

Eikenaar, C., Whitham, M., Komdeur, J., Velde, M., & Moore, I. (2011). Testosterone, Plumage Colouration and Extra-Pair Paternity in Male North-American Barn Swallows. *PloS one*, *6*, e23288.

Eliassen, S., & Kokko, H. (2008). Current analyses do not resolve whether extra-pair paternity is male or female driven. *Behavioral Ecology and Sociobiology*, *62* (11), 1795–1804.

Emlen, S. T., & Wrege, P. H. (1986). Forced Copulations and Intra-specific Parasitism: Two Costs of Social Living in the White-fronted Bee-eater. *Ethology*, *71* (1), 2–29.

Evans, M. R. (1998). Selection on swallow tail streamers. *Nature*, *394* (6690), 233–234.

Fisher, R. A. (1930). *The Genetical Theory Of Natural Selection*. At The Clarendon Press.

Forstmeier, W., Martin, K., Bolund, E., Schielzeth, H., & Kempenaers, B. (2011). Female extrapair mating behavior can evolve via indirect selection on males. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 10608–10613.

Forstmeier, W., Griffith, S., & Kempenaers, B. (2014). Female extra-pair mating: Adaptation or genetic constraint? *Trends in ecology & evolution*, *29,* 456-464.

Freeman-Gallant, C. R., Wheelwaright, N. T., Meiklejohn, K. E., States, S. L., & Sollecito, S. V. (2005). Little Effect of Extrapair Paternity on the Opportunity for Sexual Selection in Savannah Sparrows (*Passerculus Sandwichenisis*). *Evolution*, *59* (2), 422–430.

Freeman-Gallant, C. R., Taff, C. C., Morin, D. F., Dunn, P. O., Whittingham, L. A., & Tsang, S. M. (2010). Sexual selection, multiple male ornaments, and age- and condition-dependent signaling in the common yellowthroat. *Evolution; International Journal of Organic Evolution*, *64* (4), 1007–1017.

Fujita, G., & Higuchi, H. (2007). Barn swallows prefer to nest at sites hidden from neighboring nests within a loose colony. *Journal of Ethology*, *25*, 117–123.

Gibbons, D. W. (1986). Brood parasitism and cooperative nesting in the moorhen, *Gallinula chloropus*. *Behavioral Ecology and Sociobiology*, *19* (3), 221–232.

Giery, S. T. & Layman, C. A. (2015). Interpopulation variation in a condition-dependent signal: predation regime affects signal intensity and reliability. *The American Naturalist, 186* (2), 000-000.

Girndt, A., Chng, C. W. T., Burke, T., & Schroeder, J. (2018). Male age is associated with extra-pair paternity, but not with extra-pair mating behaviour. *Scientific Reports*, *8*.

Girndt, A., Cockburn, G., Sánchez-Tójar, A., Hertel, M., Burke, T., & Schroeder, J. (2019). Male age and its association with reproductive traits in captive and wild house sparrows. *Journal of Evolutionary Biology*, *32* (12), 1432–1443.

González-Solís, J., & Becker, P. H. (2002). Mounting frequency and number of cloacal contacts increase with age in common terns *Sterna hirundo*. *Journal of Avian Biology*, *33* (3), 306–310.

Gowaty, P. A. (1996). Battle of the sexes and origins of monogamy. In: Partnerships in Birds: the Study of Monogamy (*ed. Black JM*), 21-52, Oxford University Press, Oxford.

Grafen, A. (1990). Biological signals as handicaps. *Journal of Theoretical Biology, 144*, 517-546.

Gray, E. M. (1997). Do female red-winged blacbirds benefit genetically from seeking extra-pair copulations? *Animal Behaviour, 53*, 605-623.

Griffith, S. C., Owens, I. P. F., & Burke, T. (1999). Female choice and annual reproductive success favour less–ornamented male house sparrows. *Proceedings of the Royal Society of London.*

Series B: Biological Sciences, *266* (1421), 765–770.

Griffith, S. C., Owens, I. P. F., & Thuman, K. A. (2002). Extra pair paternity in birds: A review of interspecific variation and adaptive function. *Molecular Ecology*, *11*(11), 2195–2212.

Griffith, S. C., Lyon, B., & Montgomerie, R. (2004). Quasi-parasitism in birds. *Behavioral Ecology and Sociobiology, 56*, 191-200.

Griffith, S. C., Barr, I., Sheldon, B. C., Rowe, L. V., & Burke, T. (2009). Egg patterning is not a reliable indicator of intraspecific brood parasitism in the blue tit *Cyanistes caeruleus*. *Journal of Avian Biology*, *40*, 337-341.

Hamilton, W. & Zuk, M. (1982). Heritable true fitness and bright birds: a role for parasites? *Science, 218* (4570), 384-387.

Hansen, T. F., & Price, D. K. (1999). Age- and sex-distribution of the mutation load. *Genetica*, *106* (3), 251–262.

Hasegawa, M., & Arai, E. (2020). Fork tails evolved differently in swallows and swifts. *Journal of Evolutionary Biology*, *33* (7), 911–919.

Hasegawa, M., Arai, E., & Kutsukake, N. (2016). Evolution of tail fork depth in genus Hirundo. *Ecology and Evolution*, *6* (3), 851–858.

Hasselquist, D., & Sherman, P. W. (2001). Social mating systems and extrapair fertilizations in passerine birds. *Behavioral Ecology 12*, 457-466.

Hepp, G. R., Kennamer, R. A., & Harvey, W. F. (1990). Incubation as a reproductive cost in female wood ducks. *The Auk, 107 (4)*, 756-764.

Hill, G. E. & Montgomerie, R. (1994). Plumage Colour Signals Nutritional Condition in the House Finch. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 258* (1351), 47-52.

Hill, G. E. (2011). Condition-dependent traits as signals of the functionality of vital cellular processes. *Ecology Letters, 14*, 625-364.

Hsu, Y.-H., Schroeder, J., Winney, I., & Burke, T. (2015). Are extra-pair males different from cuckolded males? A case study and a meta-analytic examination. *Molecular Ecology, 24*, 1558-1571.

Huttegger, S., Bruner, J. & Zollman, K. (2015). The Handicap Principle Is an Artifact. *Philosophy of Science, 82*, 997-1009.

Iwasa, Y. & Pomiankowski, A. (1994). The evolution of mate preferences for multiple sexual ornaments. *Evolution, 483,* 853-867.

Jennions, M. D., Møller, A. P., & Petrie, M. (2001). Sexually Selected Traits and Adult Survival: A Meta-Analysis. *The Quarterly Review of Biology*, *76* (1), 3–36.

Kempenaers, B., Verheyen, G. R., & Dhondi, A. A. (1997). Extrapair paternity in the blue tit (*Parus caeruleus*): Female choice, male characteristics, and offspring quality. *Behavioral Ecology*, *8* (5), 481–492.

Kempenaers, B., Congdon, B., Boag, P., & Robertson, R. J. (1999). Extrapair paternity and egg

hatchability in tree swallows: Evidence for the genetic compatibility hypothesis? *Behavioral Ecology*, *10* (3), 304–311.

Kendra, P. E., Roth, R. R., & Tallamy, D. W. (1988). Conspecific Brood Parasitism in the House Sparrow. *The Wilson Bulletin*, *100* (1), 80–90.

Kirkpatrick, M. (1987). Sexual Selection by Female Choice in Polygynous Animals. *Annual Review of Ecology and Systematics*, *18* (1), 43–70.

Kirkpatrick, M., Price, T., & Arnold, S. (1990). The Darwin-Fisher Theory of Sexual Selection in Monogamous Birds. *Evolution*, *44*, 180–193.

Kleven, O., Jacobsen, F., Izadnegahdar, R., Robertson, R., & Lifjeld, J. (2006). Male tail streamer length predicts fertilization success in the North American Barn Swallow (*Hirundo rustica erythrogaster*). *Behavioral Ecology and Sociobiology*, *59*, 412–418.

Klvaňa, P., Cepák, J., Munclinger, P., Michálková, R., Tomášek, O. & Albrecht, T. (2018). Around the Mediterranean: an extreme example of loop migration in a long-distance migratory passerine. *Journal of Avian Biology*, *49,* jav-01595.

Kokko, H., Ranta, E., Ruxton, G., & Lundberg, P. (2002a). Sexually transmitted disease and the evolution of mating systems. *Evolution, 56*, 1091-1100.

Kokko, H., Brooks, R., McNamara, J. M., & Houston, A. I. (2002b). The sexual selection continuum. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269* (1498), 1331–1340.

Kose, M. & Møller, A. P. (1999). Sexual selection, feather breakage and parasites: the importance of white spots in the tail of the barn swallow (*Hirundo rustica*). *Behavioral Ecology and Sociobiology, 45,* 430-436.

Kotiaho, J. S. (2001). Costs of sexual traits: A mismatch between theoretical considerations and empirical evidence. *Biological Reviews of the Cambridge Philosophical Society*, *76* (3), 365–376.

Kruuk, L., Merilä, J., & Sheldon, B. (2001). Phenotypic Selection on a Heritable Size Trait Revisited. *The American Naturalist*, *158*, 557–571.

Lack, D. (1968). *Ecological Adaptations for Breeding in Birds.* Methuen Ltd, London.

Laskemoen, T., Albrecht, T., Bonisoli-Alquati, A., Cepak, J., de Lope, F., Hermosell, I. G., Johannessen, L. E., Kleven, O., Marzal, A., Mousseau, T. A., Møller, A. P., Robertson, R. J., Rudolfsen, G., Saino, N., Vortman, Y., & Lifjeld, J. T. (2013). Variation in sperm morphometry and sperm competition among barn swallow (*Hirundo rustica*) populations. *Behavioral Ecology and Sociobiology*, *67* (2), 301–309.

Lifjeld, J. T., Kleven, O., Jacobsen, F., McGraw, K. J., Safran, R. J., & Robertson, R. J. (2011). Age before beauty? Relationships between fertilization success and age-dependent ornaments in barn swallows. *Behavioral Ecology and Sociobiology*, *65* (9), 1687–1697.

Lifjeld, J. T., Gohli, J., Albrecht, T., Garcia-del-Rey, E., Johannessen, L. E., Kleven, O., Marki, P. Z.,

Omotoriogun, T. C., Rowe, M. & Johnsen, A. (2019). Evolution of female promiscuity in Passerides songbirds. *BMC Evolutionary Ecology, 19*, 169.

Lombardo, M. P., Power, H. W., Stouffer, P. C., Romagnano, L. C., & Hoffenberg, A. S. (1989). Egg removal and intraspecific brood parasitism in the European starling (*Sturnus vulgaris*). *Behavioral Ecology and Sociobiology*, *24* (4), 217–223.

Lyon, B. E., Hochachka, W. M., & Eadie, J. M. (2002). Paternity-parasitism trade-offs: A model and test of host-parasite cooperation in an avian conspecific brood parasite. *Evolution; International Journal of Organic Evolution*, *56* (6), 1253–1266.

Lyon, B., & Eadie, J. (2008). Conspecific Brood Parasitism in Birds: A Life-History Perspective. *Annual Review of Ecology Evolution and Systematics*, *39*, 343–363.

Lyu, N., Servedio, M. R., & Sun, Y.H. (2018). Nonadaptive female pursuit of extrapair copulations can evolve through hitchhiking. *Ecology and Evolution*, *8* (7), 3685–3692.

Mays, H. L., & Hill, G. E. (2004). Choosing mates: Good genes versus genes that are a good fit. *Trends in Ecology & Evolution*, *19* (10), 554–559.

Mays, H. L., Albrecht, T., Liu, M. & Hill, G. E. (2008). Female choice for genetic complementarity in birds: a review. *Genetica 134*, 147–158.

McGraw, K. J., Mackillop, E. A., Dale, J. & Hauber, M. E. (2002). Different colors reveal different information: how nutritional stress affects the expression of melanin- and structurally based ornamental plumage. *Journal of Experimental Biology, 205*, 3747-3755.

Møller, A. P. (1987a). Mate guarding in the swallow *Hirundo rustica*: An experimental study. *Behavioral Ecology and Sociobiology*, *21* (2), 119–123.

Møller, A. P. (1987b). Intraspecific nest parasitism and anti-parasite behaviour in swallows, *Hirundo rustica*. *Animal Behaviour*, *35,* 247-254.

Møller, A. P. (1987c). Variation in badge size in male house sparrows *Passer domesticus*: Evidence for status signalling. *Animal Behaviour*, *35* (6), 1637–1644.

Møller, A. P. (1988a). Female choice selects for male sexual tail ornaments in the monogamous swallow. *Nature*, *332* (6165), 640–642.

Møller, A. P. (1988b). Badge size in the house sparrow *Passer domesticus*. *Behavioral Ecology and Sociobiology*, *22* (5), 373–378.

Møller, A. P. (1989). Natural and sexual selection on a plumage signal of status and on morphology in house sparrows, *Passer domesticus*. *Journal of Evolutionary Biology*, *2* (2), 125–140.

Møller, A. P. (1991). Viability is positively related to degree of ornamentation in male swallows. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *243* (1307), 145–148.

Møller, A. P. (1994a). Sexual Selection and the Barns Swallow. Oxford University Press, Oxford.

Møller, A. P. (1994b). Male ornament size as a reliable cue to enhanced offspring viability in the barn swallow. *Proceedings of the National Academy of Sciences of the United States of America*, *91*

(15), 6929–6932.

Møller, A. & Pomiankowski, A. (1993). Why have birds got multiple sexual ornaments? *Behavioral Ecology and Sociobiology, 32*, 167-176.

Møller, A. P. & de Lope, F. (1994). Differential costs of a secondary sexual character: an experimental test of the handicap principle. *Evolution, 48*, 1676-1683.

Møller, A. P. & Tegelström, H. (1997). Extra-pair paternity and tail ornamentation in the barn swallow *Hirundo rustica. Behavioral Ecology and Sociobiology, 41*, 353-360.

Møller, A. P., Barbosa, A., Cuervo, J. J., de Lope, F., Merino, S. & Saino, N. (1998). Sexual selection and tail streamers in the barn swallow. *Proceedings of the Royal Society B: Biological Sciences, 265*, 409-414.

Møller, A. P., Brohede, J., Cuervo, J. J., de Lope, F., & Primmer, C. (2003). Extrapair paternity in relation to sexual ornamentation, arrival date, and condition in a migratory bird. *Behavioral Ecology*, *14* (5), 707–712.

Nielsen, C. R., Semel, B., Sherman, P. W., Westneat, D. F., & Parker, P. G. (2006). Host-parasite relatedness in wood ducks: Patterns of kinship and parasite success. *Behavioral Ecology*, *17* (3), 491–496.

Neuman, C. R., Safran, R. J., & Lovette, I. J. (2007). Male tail streamer length does not predict apparent or genetic reproductive success in North American barn swallows *Hirundo rustica erythrogaster*. *Journal of Avian Biology, 38*, 28-36.

Norberg, R. Å. (1994). Swallow tail streamer is a mechanical device for self-deflection of tail leading edge, enhancing aerodynamic efficiency and flight manoeuvrability. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *257* (1350), 227–233.

Ohlsson, T., Smith, H. G., Råberg, L., & Hasselquist, D. (2002). Pheasant sexual ornaments reflect nutritional conditions during early growth. *Proceedings of the Royal Society B: Biological Sciences*, *269*, 21–27.

Owens, I. P. F., & Hartley, I. R. (1998). Sexual dimorphism in birds: Why are there so many different forms of dimorphism? *Proceedings of the Royal Society B: Biological Sciences*, *265* (1394), 397–407.

Park, K. J., Evans, M. R., & Buchanan, K. L. (2000). Assessing the aerodynamic effects of tail elongations in the house martin (*Delichon urbica*): Implications for the initial selection pressures in hirundines. *Behavioral Ecology and Sociobiology*, *48* (5), 364–372.

Payne, R. B. (1977). The ecology of brood parasitism in birds. *Annual Review of Ecology, Evolution, and Systematics, 8*, 1-28.

Petrie, M. (1986). Reproductive strategies of male and female moorhens (*Gallinula chloropus*). In: Rubenstein DI, Wrangham RW (eds) Ecological aspects of social evolution. Princeton University Press, Princeton, N.J., pp 43–63.

Penn, D. & Számadó, S. (2020). The Handicap Principle: how an erroneous hypothesis became a scientific principle. *Biological Reviews,* 95, 267-290.

Petrie, M., & Møller, A. P. (1991). Laying eggs in others' nests: Intraspecific brood parasitism in birds. *Trends in Ecology & Evolution*, *6* (10), 315–320.

Petrie, M., & Kempenaers, B. (1998). Extra-pair paternity in birds: Explaining variation between species and populations. *Trends in Ecology & Evolution*, *13* (2), 52–58.

Romano, A., Costanzo, A., Rubolini, D., Saino, N., & Møller, A. P. (2017a). Geographical and seasonal variation in the intensity of sexual selection in the barn swallow *Hirundo rustica*: A meta-analysis. *Biological Reviews*, *92*, 1582-1600.

Romano, A., Saino, N., & Møller, A. P. (2017b). Viability and expression of sexual ornaments in the barn swallow *Hirundo rustica*: A meta-analysis. *Journal of Evolutionary Biology*, *30* (10), 1929–1935.

Rowe, L. V., Evans, M. R., & Buchanan, K. L. (2001). The function and evolution of the tail streamer in hirundines. *Behavioral Ecology*, *12* (2), 157–163.

Ruxton, G. D., & Broom, M. (2002). Intraspecific brood parasitism can increase the number of eggs that an individual lays in its own nest. *Proceedings of the Royal Society B: Biological Sciences*, *269* (1504), 1989–1992.

Saetre, G. P., Fossnes, T., & Slagsvold, T. (1995). Food Provisioning in the Pied Flycatcher: Do Females Gain Direct Benefits from Choosing Bright-Coloured Males? *Journal of Animal Ecology*, *64* (1), 21–30.

Sánchez-Tójar, A., Nakagawa, S., Sánchez-Fortún, M., Martin, D. A., Ramani, S., Girndt, A., Bókony, V., Kempenaers, B., Liker, A., Westneat, D. F., Burke, T., & Schroeder, J. (2018). Meta-analysis challenges a textbook example of status signalling and demonstrates publication bias. *eLife*, *7*, e37385.

Safran, R. J., & McGraw, K. J. (2004). Plumage coloration, not length or symmetry of tail-streamers, is a sexually selected trait in North American barn swallows. *Behavioral Ecology*, *15* (3), 455–461.

Safran, R. J., Neuman, C. R., McGraw, K. J., & Lovette, I. J. (2005). Dynamic Paternity Allocation as a Function of Male Plumage Color in Barn Swallows. *Science, 309,* 2220–2212.

Safran, R. J., Vortman, Y., Jenkins, B. R., Hubbard, J. K., Wilkins, M. R., Bradley, R. J., & Lotem, A. (2016). The maintenance of phenotypic divergence through sexual selection: An experimental study in barn swallows *Hirundo rustica. Evolution, 70* (9), 2074–2084.

Saino, N., Primmer, C. R., Ellegren, H., & Møller, A. P. (1997a). An Experimental Study of Paternity and Tail Ornamentation in the Barn Swallow (*Hirundo rustica*). *Evolution*, *51* (2), 562–570.

Saino, N., Bolzern, A. M., & Møller, A. P. (1997b). Immunocompetence, ornamentation, and viability of male barn swallows (*Hirundo rustica*). *Proceedings of the National Academy of Sciences*, *94* (2), 549–552.

Saino, N., Primmer, C. R., Ellegren, H., & Møller, A. P. (1999). Breeding synchrony and paternity in the barn swallow (*Hirundo rustica*). *Behavioral Ecology and Sociobiology, 45,* 211-218.

Saino, N., Romano, M., Romano, A., Rubolini, D., Ambrosini, R., Caprioli, M., Parolini, M., Scandolara, C., Bazzi, G. & Costanzo, A. (2015). White tail spots in breeding Barn Swallows *Hirundo rustica* signal body condition during winter moult. *Ibis, 1574*, 722-730.

Scordato, E. S., & Safran, R. J. (2014). Geographic variation in sexual selection and implications for speciation in the Barn Swallow. *Avian Research*, *5* (1), 8.

Semel, B., & Sherman, P. (2001). Intraspecific parasitism and nest-site competition in wood ducks. *Animal Behaviour*, *61*, 787–803.

Shaw, R. C., & Hauber, M. E. (2009). Experimental support for the role of nest predation in the evolution of brood parasitism. *Journal of Evolutionary Biology*, *22* (6), 1354–1358.

Scheuber, H., Jacot, A., & Brinkhof, M. W. (2003). Condition dependence of a multicomponent sexual signal in the field cricket *Gryllus campestris*. *Animal Behaviour*, *65*, 721–727.

Schlicht, E., & Kempenaers, B. (2013). Effects of social and extra-pair mating on sexual selection in blue tits (*Cyanisteus caeruleus*). *Evolution, 67* (5), 1420–1434.

Simmons, R., & Scheepers, L. (1996). Winning by a Neck: Sexual Selection in the Evolution of Giraffe. *The American Naturalist*, *148*, 771–786.

Smith, H. G., Montgomerie, R., Poldman, T., White, B. N., & Boag, P. T. (1991). DNA fingerprinting reveals relation between tail ornaments and cuckoldry in barn swallows, *Hirundo rustica*. *Behavioral Ecology*, *2* (1), 90–98.

Smith, Henrik G., & Montgomerie, R. (1991). Sexual selection and the tail ornaments of North American barn swallows. *Behavioral Ecology and Sociobiology*, *28* (3), 195–201.

Sorenson, M. D. (1992). Why is conspecific nest parasitism more frequent in waterfowl than in other birds – comment. *Canadian Journal of Zoology 109*, 272-292.

Soulsbbury, C. D., Lipponen, A., Wood, K., Mien, CH. A., Hoffman, J. I., & Lebigre, C. (2018). Age- and quality-dependent DNA methylation correlate with melanin-based coloration in a wild bird. *Ecology and Evolution, 8,* 6547-6557.

Spottiswoode, C., & Møller, A. P. (2004). Extrapair paternity, migration, and breeding synchrony in birds. *Behavioral Ecology 15*, 41-57.

Stutchbury, B. J. M., & Morton, E. S. (2001). Behavioral ecology of tropical birds. San Diego, Academic.

Sundberg, J., & Dixon, A. (1996). Old, colourful male yellowhammers, *Emberiza citrinella*, benefit from extra-pair copulations. *Animal Behaviour*, *52* (1), 113–122.

Suter, S. M., Keiser, M., Feignoux, R., & Meyer, D. R. (2007). Reed bunting females increase fitness through extra-pair mating with genetically dissimilar males. *Proceedings of the Royal Society B: Biological Sciences*, *274* (1627), 2865–2871.

Tarof, S. A., Kramer, P. M., Tautin, J., & Stutchbury, B. J. M. (2012). Effects of known age on male

paternity in a migratory songbird. *Behavioral Ecology*, *23* (2), 313–321.

Thompson, C. W., Hillgarth, N., Leu, M., & McClure, H. E. (1997). High parasite load in house finches (*Carpodacus mexicanus*) is correlated with reduced expression of a sexually selected trait. *American Naturalist*, *149*, 270–294.

Thusius, K. J., Peterson, K. A., Dunn, P. O., & Whittingham, L. A. (2001). Male mask size is correlated with mating success in the common yellowthroat. *Animal Behaviour*, *62* (3), 435–446.

Tregenza, T., & Wedell, N. (2000). Genetic compatibility, mate choice and patterns of parentage: Invited review. *Molecular Ecology*, *9* (8), 1013–1027.

Trivers, R. L. (1972). Parental Investment and Sexual Selection. In *Sexual Selection and the Descent of Man* 136-179, Aldine Publishing Company, Chicago.

Turner, A. (2006). The barn swallow. T. & A.D. Poyser, London.

Vedder, O., Komdeur, J., van der Velde, M., Schut, E., & Magrath, M. J. L. (2011). Polygyny and extra-pair paternity enhance the opportunity for sexual selection in blue tits. *Behavioral Ecology and Sociobiology*, *65* (4), 741–752.

Veiga, J. P. (1993). Badge Size, Phenotypic Quality, and Reproductive Success in the House Sparrow: A Study on Honest Advertisement. *Evolution*, *47* (4), 1161–1170.

Verhulst, C. E., Mateman, A. C., Zwier, M. V., Caro, S. P., Verhoeven, K. J. F., & van Oers, K. (2016). Evidence from pyrosequencing indicates that natural variation in animal personality is associated with DRD4 DNA methylation. *Molecular Ecology*, *25*, 1801–1811.

Vortman, Y., Lotem, A., Dor, R., Lovette, I., & Safran, R. J. (2011). The sexual signals of East-Mediterranean barn swallow: A different swallow tale. *Behavioral Ecology, 22* (6), 1344-1352.

Vortman, Y., Lotem, A., Dor, R., Lovette, I., & Safran, R. J. (2013). Multiple Sexual Signals and Behavioral Reproductive Isolation in a Diverging Population. *The American Naturalist*, *182* (4), 514–523.

Wakano, J., & Ihara, Y. (2005). Evolution of Male Parental Care and Female Multiple Mating: Game-Theoretical and Two-Locus Diploid Models. *The American Naturalist*, *166*, E32-44.

Walker, L., Stevens, M., Karadas, F., Kilner, R. & Ewen, J. (2013). A window on the past: male ornamental plumage reveals the quality of their early-life environment. *Proceedings of the Royal Society B: Biological Sciences, 280* (1756), 20122852-20122852.

Weatherhead, P. J., & Boag, P. T. (1995). Pair and extra-pair mating success relative to male quality in red-winged blackbirds. *Behavioral Ecology and Sociobiology*, *37* (2), 81–91.

Webster, M. S., Pruett-Jones, S., Westneat, D. F., & Arnold, S. J. (1995). Measuring the effects of pairing success, extra-pair copulations and mate quality on the opportunity for sexual selection. *Evolution*, *49* (6), 1147–1157.

Webster, M. S., Chuang-Dobbs, H. C., & Holmes, R. T. (2001). Microsatellite identification of extrapair sires in a socially monogamous warbler. *Behavioral Ecology*, *12* (4), 439–446.

Webster, M., Tarvin, K., Tuttle, E., & Pruett-Jones, S. (2007). Promiscuity drives sexual selection in a monogamous bird. *Evolution, 61*, 2205–2211.

Westneat, D. F., Sherman, P. W., & Morton M. (1990). The ecology and evolution of extra-pair copulations in birds. *Current ornithology*, *7*, 331–369.

Westneat, D. F., & Sargent, C. R. (1996). Sex and parenting: The effects of sexual conflict and parentage on parental strategies. *Trends in Ecology & Evolution*, *11* (2), 87–91.

Westneat, D. F., & Stewart, I. (2003). Extra-Pair Paternity in Birds: Causes, Correlates, and Conflict. *Annual Review of Ecology, Evolution, and Systematics*, *34*, 365–396.

Wetton, J. H., & Parkin, D. T. (1991). An association between fertility and cuckoldry in the house sparrow *Passer domesticus*. *Proccedings of the Royal Society of London, series B, Biological Sciences, 245*, 227-233.

Whittingham, L. A., & Dunn, P. O. (2005). Effects of extra-pair and within-pair reproductive success on the opportunity for selection in birds. *Behavioral Ecology*, *16* (1), 138–144.

Wilkins, M. R., Karaardıç, H., Vortman, Y., Parchman, T. L., Albrecht, T., Petrželková, A., Özkan, L., Pap, P. L., Hubbard, J. K., Hund, A. K., & Safran, R. J. (2016). Phenotypic differentiation is associated with divergent sexual selection among closely related barn swallow populations. *Journal of Evolutionary Biology*, *29* (12), 2410–2421.

Williams, G. C. (1975). Sex and Evolution. *Princeton University Press*, New Jersey.

Wrege, P. H., & Emlen, S. T. (1987). Biochemical determination of parental uncertainty in white-fronted bee-eaters. *Behavioral Ecology and Sociobiology*, *20* (3), 153–160.

Yin, Y., Morgunova, E., Jolma, A., Kaasinen, E., Sahu, B., Khund-Sayeed, S., Das, P. K., Kivioja. T., Dave, K., Zhong, F., Nitta, K. R., Taipale, M., Popov, A., Ginno, P. A., Domcke, S., Yan, J., Schübeler, D., Vinson, CH., & Taipale, J. (2017). Impact of cytosine methylation on DNA binding specificities of human transcription factors. *Science*, *356*, eaaj2239.

Yom-Tov, Y. (1980). Intraspecific Nest Parasitism in Birds. *Biological Reviews*, *55* (1), 93–108.

Yom-Tov, Y. (2001). An updated list and some comments on the occurrence of intraspecific nest parasitism in birds. *Ibis*, *143* (1), 133–143.

Yom-Tov, Y. & Geffen, E. (2006). On the origin of brood parasitism in altricial birds. *Behavioral Ecology, 17*, 196-205.

Zahavi, A. (1975). Mate selection – A selection for a handicap. *Journal of Theoretical Biology 53* (1), 205-214.

Publikace 1

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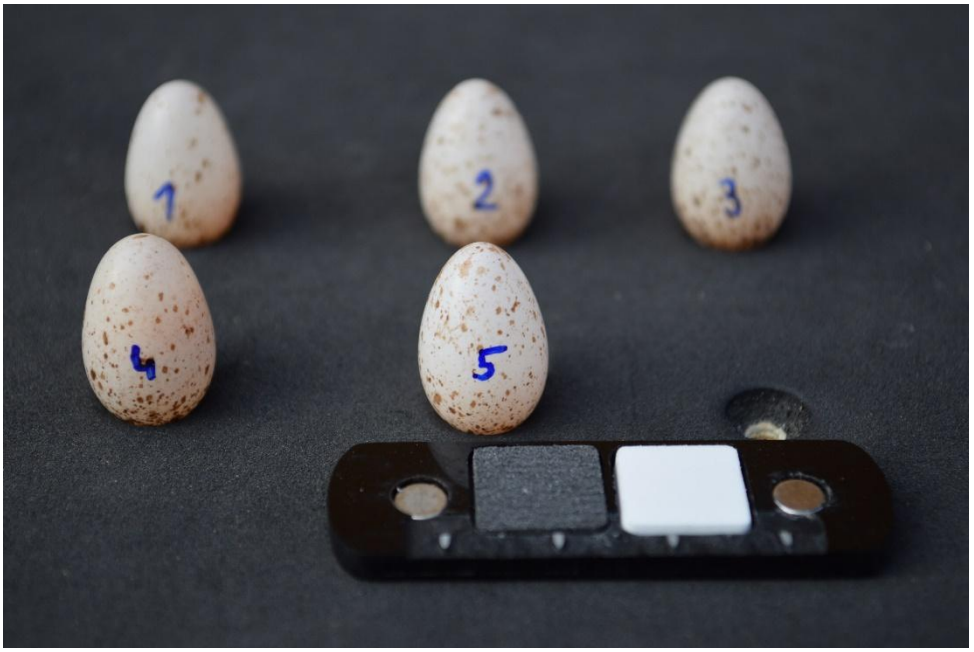

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ORIGINAL PAPER

Brood parasitism and quasi-parasitism in the European barn swallow *Hirundo rustica rustica*

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Abstract We studied patterns of extra-pair maternity (EPM) in 245 nests (225 nests belonging to 120 females of known identity) of sexually promiscuous European barn swallows (*Hirundo rustica rustica*) over a 3-year period. At least one EPM nestling was identified in 54 nests (22.0 %), representing 5.7 % of a total of 1060 nestlings. Up to 28.3 % of all EPM nestlings resulted from quasi-parasitism (QP), whereby nest-attending males sired parasitic offspring. Nests of quasi-parasitic females were never in close proximity to the host nest. Our data thus indicate nonrandom QP patterns in our population suggesting that QP can be considered a third alternative reproductive strategy alongside extra-pair paternity (EPP) and intraspecific brood parasitism (IBP). Of several socioecological factors evaluated, only number of simultaneous egg-laying females in the population proved a good predictor for EPM occurrence. Whereas parasitic females produced more offspring per breeding attempt than was the population average, both QP and IBP affected host female reproductive output, being associated with a reduced number of her offspring produced from the nest. On the contrary, QP resulted in an increase in the number of offspring produced by nest-attending males, suggesting that males may benefit from cooperating with parasitic females at the expense of their social partners.

Keywords Altricial birds · Colonial breeding · Conspecific brood parasitism · Egg dumping · Host fitness · Parasite fitness

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Introduction

Intraspecific brood parasitism (IBP), whereby parasitic females increase their fitness by reducing their own parental investment at the expense of a host, can be viewed as an alternative female reproductive strategy in egg-laying species (Andersson 1984; Eadie 1989). Females adopting an IBP strategy typically spread their eggs in nests of other females of the same species and provide no subsequent parental care. Several explanations for the adaptive function of IBP have been suggested. The first proposes that parasitic laying is employed when typical nesting is impossible or unprofitable (making the "best-of-a-bad-situation"; e.g. Dawkins 1980). An alternative, the "enhancement hypothesis" (Kendra et al. 1988), suggests that parasitic egg-laying leads to an improvement in reproductive output, even when conditions for nesting are ideal (see also Åhlund and Andersson 2001).

Since conspecific parasitic eggs and young may not show morphological differences from the host's own eggs and offspring, the occurrence of extra-pair maternity (EPM) resulting from IBP is often difficult to detect in avian populations;

hence, traditional methods used to detect parasitic events, such as abnormal clutch size or unusual laying sequence (Yom-Tov 1980; MacWhirter 1989), may underestimate the rate of parasitism (Andersson and Åhlund 2001; Griffith et al. 2009). As a result, until relatively recently (Andersson and Åhlund 2001; Yom-Tov 2001; Arnold and Owens 2002), IBP was considered rare in birds compared to interspecific brood parasitism (Payne 1977).

Based on available data, IBP appears to be more common in precocial (rather than altricial) avian species and appears to be particularly rare in songbirds, where it has been documented in only ~1 % of species (Yom-Tov 2001), often colonial breeders or cavity nesters (e.g., Brown 1984; Møller 1987; Lombardo et al. 1989; Jackson 1993). Explanations for this include variation in life histories and the associated costs of parasitism to the host parents. Unlike precocial birds, altricial species typically have relatively small clutches, start incubation during egg-laying, and provide more parental care to the offspring (Ar and Yom-Tov 1978; Andersson 1984; Sayler 1992; Sorenson 1992; Lyon and Everding 1996). In altricial species, both the number and quality of host offspring could be affected by IBP due to intense intranest competition (Brown and Brown 1991), while enlarged clutches in both precocial and altricial species may suffer from incubation failure (Eadie 1989; Semel and Sherman 2001) or increased risk of predation due to the need for longer incubation (Gibbons 1986; Nielsen et al. 2006). Moreover, host fitness may be reduced (Lombardo et al. 1989) through damage or removal of the host's own eggs by the parasitic female.

Several socioecological factors have been suggested as affecting rates of IBP in birds. At interspecific levels, IBP seems to be particularly common in species with limited breeding opportunities (typically cavity nesters) or in species where nests are easily detected by the conspecific parasite (e.g., Semel and Sherman 2001; Hoi et al. 2010). Breeding density is a further crucial factor determining occurrence of IBP (Bennett and Owens 2002) as high breeding density will increase the number of neighboring nests available to the parasite (Eadie et al. 1988). Density dependence of parasitism rates has also been demonstrated at the intraspecific level in some species using both molecular based (Waldeck et al. 2004) and observational (Møller 1987; Brown and Brown 1989) approaches. In a similar manner, high breeding synchrony could also promote IBP since synchronous laying offers greater availability of nests to the egg-laying parasite (Rohwer and Freeman 1989).

In addition to IBP, EPM can also result from quasi-parasitism (QP). QP occurs in genetically promiscuous systems where parasitic eggs are fertilized by the resident male (Emlen and Wrege 1986), such that the offspring are the progeny of the attending male but not of the attending female (Griffith et al. 2004). The adaptive function of this behavior in avian populations is discussed by Lyon and Eadie (2008). QP has been detected in only a few avian species and mostly at low frequencies, leading to the hypothesis that QP arises by chance (reviewed in Griffith et al. 2004). Specifically, the hypothesis predicts that QP frequencies simply reflect the likelihood that a parasitic egg is fertilized by a nest attending male by chance in systems with high levels of EPP (Griffith et al. 2004). Alternatively, nonrandom distribution of QP in a population may suggest that this is an adaptive strategy (Alves and Bryant 1998; Berger et al. 2013). The socioecological correlates of QP in birds remain largely unexplored.

Here, we use molecular tools to detect occurrence of EPM offspring in a small altricial passerine bird, the European barn swallow (*Hirundo rustica rustica*). Barn swallows are socially monogamous with moderate-to-high levels of genetic promiscuity (Saino et al. 1997; Safran et al. 2005; Laskemoen et al. 2013). They typically breed in colonies, though some pairs do breed solitarily (Møller 1987; Fujita and Higuchi 2007). Although IBP has been documented in this species (Møller 1987), a molecular-based approach for identifying EPM nestlings has rarely been adopted (but see Møller et al. 2003). Based on observational data, it has been suggested that IBP rate increases with colony size in barn swallows and that females nesting solitarily (Møller 1987), or at hidden sites (Fujita and Higuchi 2007), escape parasitism. Furthermore, data for several swallow species indicate that there appear to be fitness costs to the host female in terms of clutch reduction associated with EPM (Brown 1984; Møller 1987). Despite intensive molecular-based studies of parentage in barn swallows (e.g., Kleven et al. 2005; Safran et al. 2005), QP has only been reported for one barn swallow population to date (Møller et al. 2003). There are some uncertainties concerning this finding, however, as no other EPM was simultaneously detected (see Griffith et al. 2004 for discussion). The main objectives of this study are (1) to assess rates of EPM in a Central European population of barn swallows and to evaluate (a) whether EPM is distributed randomly across the breeding season or (b) is randomly distributed in relation to the number of potential breeding parasites available; (2) to analyze the extent to which risk of parasitism in barn swallows is associated with solitary vs. colonial breeding; (3) to estimate potential fitness costs to the hosts arising from EPM (IBP and QP) using molecular determination of EPM eggs and a large sample of nests; (4) to assess the consequences of QP on the nest-attending male; and (5) to evaluate the hypothesis that QP occurs in this genetically promiscuous species as "chance QP" (e.g., Alves and Bryant 1998).

Methods

Study species and study area The barn swallow is a socially monogamous passerine that usually breeds in colonies. Females usually lay one to six eggs (one egg usually being

laid each day; AP and TA, unpublished data) and start to incubate after the penultimate or ultimate egg has been laid (Møller 1994). The study was completed within the Třeboňsko Protected Landscape Area in South Bohemia (Doudlebia; Czech Republic). Barn swallow nests were identified at two isolated farms (Šaloun, Lomnice nad Lužnicí [49° 4′ 7.762″ N, 14° 42′ 36.521″ E] and Hamr, Lužnice [49° 3′ 25.288″ N, 14° 46′ 10.82″ E]) from May to August between 2010 and 2012.

Field procedures In both study areas, barn swallow colonies were located in rural buildings such as barns and cow sheds, with just a few solitary pairs breeding in buildings not directly associated with agriculture. A nest was considered as solitary when no other simultaneously occupied nest was located in the same room/barn. Each site hosted from 20 to 40 breeding swallow pairs each year. Note that we were unable to follow second clutches in 2011 due to logistic problems. As a result, the number of inspected nests varies between ~50 and 120 each year (Table S1). Swallows were systematically captured using a mist-net on several occasions over the breeding season, and each individual was tagged with an aluminum ring (National Museum of Prague) and marked with a unique color combination of 1–2 colored plastic rings (AVINET). Of the 40 females trapped during their egg-laying period, EPM was detected in seven, while EPM was detected in 41 of the 181 females trapped outside that period. Overall, there was no significant difference between the groups (Chi-squared test, χ^2=0.83, df=1, p=0.36). Where possible, males and females were followed in order to identify the position of their nest. A venipuncture blood sample (~20 μl) was taken from all adult birds. Individuals were sexed according to the shape of the cloacal protuberance (Svensson 1984), assignment later being confirmed through inspection for presence (female) or absence (male) of an incubation patch and through incubation behaviour (only females incubate in the European subspecies; Møller 1994). Most nests were found during the egg-laying period (first and second breeding attempts) and were subsequently checked at 2- to 5-day intervals. At 8 days of age, chicks were ringed and a blood sample taken from the brachial vein. All blood samples (from chicks and adults) were stored in 96 % ethanol. Brood size and reproductive output were estimated as the number of 8-day-old nestlings present in the nest. Although the number of fledglings produced from each nest could have declined, partial nest mortality is rare in barn swallows and, despite the finding of 65 unhatched eggs in nests (see below), there was a positive relationship between clutch size and brood size in our population (Spearman's rank correlation, r_s=0.84, n=245, p<0.001). Moreover, mortality is most often due to factors that are likely to be independent of the EPM status of the offspring (e.g., predation). We distinguish between the apparent brood size (the number of offspring present) and the realized brood size (the number of offspring genetically related to the nest attending parents). For brood parasitic females, the total reproductive output was measured as the number of 8-day-old offspring found in their own and in the hosts' nests. Unhatched eggs (n=65) were taken from the nests and stored in ethanol for further DNA analysis (see below). Over the 2010 to 2012 study period, barn swallows initiated 245 successful breeding attempts (66 nests in 2010, 59 nests in 2011, and 120 nests in 2012; Table S1) in our study plots. Nest initiation date was estimated either directly, by observing the appearance of the first egg laid in the clutch, or extrapolated based on the assumption that one egg is laid each day in a clutch of given size. Although the latter assumption can be violated due to the occurrence of egg dumping, only one EPM offspring usually occurred in a brood (see below), indicating that bias was not high. For each nest (breeding female), we calculated the number of potential parasites available as the number of females in the population (each breeding colony treated separately) displaying synchronous egg-laying with the focal female (i.e., with at least partly overlapping egg-laying periods).

Genotyping Blood samples were dried and DNA extracted using the DNeasy® Blood and Tissue kit (Qiagen). DNA concentration was approximately 10 ng/μl. Nestlings and adults were genotyped at six highly-polymorphic microsatellite autosomal loci previously developed for barn swallows: Hir6, Hir10, Hir15, Hir20, Hir22 (Tsyusko et al. 2007; amplified in a single multiplex reaction hereinafter referred to as PCR 1) and HrU10 (Primmer et al. 1995; amplified in a separate reaction hereinafter referred to as PCR 2). Forward primers were fluorescently labeled, and Multiplex PCR kit (Qiagen) was used in PCR reactions. Primer concentration and labeling are given in Table S2. Reaction conditions were 15 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 90 s at 56 °C, and 60 s at 72 °C, with a final extension of 30 min at 60 °C. The PCR products were pooled (0.6-μl product of PCR 1 with 1.2-μl product of PCR 2), and mixed with GeneScan™-500 Liz® Size Standard (Applied Biosystems, 0.3 μl per sample) and formamide (12 μl per sample). The mixtures were denatured for 5 min at 95 °C, cooled on ice, and analyzed using an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). Genotypes were scored using GeneMarker® version 1.9 software (Softgenetics), with binning estimated using the FlexiBin program (Amos et al. 2007). Cervus version 3.0.3 (Field Genetics Ltd.) was used to calculate observed and expected heterozygosity, probability of exclusion, and frequency estimates of null alleles for each locus (Kalinowski et al. 2007; Table S2). The combined nonexclusion probability of the marker set was 6.33×10^{-3} for the first parent and 4.75×10^{-4} for the second parent. The Micro-Checker software program was used to test for possible scoring errors due to allelic dropout or stuttering (van Oosterhout et al. 2004). In total, 1658 individuals were genotyped at all six loci and seven at five

loci. Individuals who could not be genotyped at five loci were excluded from further analysis (two females, one male, and one chick). One hundred and fifty-two adult birds were sampled over multiple seasons and were subsequently genotyped repeatedly. Repeat genotypes were used to assess the probability of genotyping error. Identical genotypes were found in 140 (92.1 %) cases, 11 individuals (7.2 %) differed at one locus, and one bird (0.7 %) differed at two loci. Overall, average genotyping error per locus was 0.006. Significance of parentage assignment was assessed using the observed Delta statistics value with the strict 95 % confidence criterion selected (see below). Critical delta values were computed using 10,000 simulations, based on the distribution of allele frequencies and estimated genotyping error of 1 % observed in our population. We assumed that 80 % of breeding females and 90 % of males were sampled.

Parentage assignment Parentage assignment was undertaken using Cervus version 3.0.3 (Kalinowski et al. 2007) and Colony version 2.0 (Wang 2004) software. First, we carried out maternity analysis in Cervus, i.e., log-likelihood statistics were computed for all possible offspring and candidate-mother pairs (hereafter LOC). The best candidate mother was considered the genetic mother of a given chick where Delta statistics (i.e., LOC difference between the most likely and second most likely mother) was significant and indicated (a) full compatibility or (b) one mismatch in the offspring-putative mother genotype comparison that could be ascribed to the presence of null alleles, allelic drop-out (i.e., the putative female and brood parasitism [EPM] offspring were heterozygotes at that locus) or a shift of 2–4 base pairs. Each chick was typically matched with just one mother on all loci. Second, we carried out a paternity analysis using the identity of the mother obtained from the previous step. In each case, the most probable male was assigned to each chick. Males were considered the genetic father only when they showed no, or just one, mismatch and the Delta statistics value was significant. When a social father was assigned to a particular nest using behavioral observations (i.e., male repeatedly observed feeding young at the nest; 206 nests, 84.1 %), distinguishing between within-pair and extra-pair paternity (EPP) was straightforward. The social father remained unidentified in 39 nests, within which mixed paternity (young assigned to more than one genetic father) was detected in 11 nests. Since it was impossible to distinguish which of the genetic fathers was the social father, we excluded these nests from the EPP frequency calculation. We also carried out a parent-pair analysis with known sexes. This confirmed the previous assignments based on maternity and paternity analysis and, in three cases, enabled us to identify a genetic mother for chicks where the female was unassigned after the first step. In nests where we identified the social mother using field observations (72 nests), a chick was considered parasitic when it did not match the genotype of its putative mother at two or more loci. In nests where the social mother was not identified, we followed one of three scenarios:

(1) Genetic mothers were identified for all young in the nest using maternity analysis (140 nests). For the majority of chicks, we assumed that the genetic mother was also the social mother. Offspring were considered as resulting from EPM when maternity analysis suggested a different genetic mother to the social mother or when the social mother's genotype did not match at two or more loci.
(2) The social mother was identified for most chicks in the focal nest, but a single chick did not match either the social mother's, or any other female's genotype at three or more loci. These chicks were considered parasitic with an unknown parasitic mother (13 cases).
(3) The social mother at a given nest was not identified (the female was probably not caught or genotyped; 20 nests). EPM occurred in three of the 20 nests, i.e., one chick in each of the three nests matched just one candidate mother on all loci, while the others had mismatches with genotypes of all possible candidate mothers (see Table S3). The genetic mother of a parasitic chick, or the genetic father of a QP chick, was assigned only when the genotype of the putative parent was compatible with the offspring genotype at all six loci. We used such strict criteria to determine parasitic females and QP males in order to avoid any identification error; hence, the results show minimum estimates of EPP and QP frequency. For further analysis of nests where the social mother was unidentified, we performed several runs with the Colony software program. The results were consistent with those from the Cervus program. In nests where EPM was not detected by Cervus, all young were identified as full siblings or half siblings (in the case of EPP offspring detected by Cervus) in the Colony runs. In three nests where EPM was detected using Cervus, the presumably parasitic chick was shown to be unrelated to its nest mates using Colony. One chick was considered as EPP offspring using Cervus; however, as Colony showed it to be unrelated to its nest mates, we finally treated it as a parasitic chick. In two other nests where neither the social female nor the social male were identified, two chicks (one from each nest) proved to be half siblings of their nest mates. Since prevalence of EPP is more frequent than QP in our population, we treated these chicks as offspring resulting from EPP.

Genotypes were successfully obtained for 1060 young and 605 adults (271 females and 334 males), while genetic mothers were identified for 972 young (91.7 %). In 20 nests (86 young), the social female was not identified. Of 972

offspring where the mother was identified, 899 matched the genetic mother's genotype (92.5 %) at all six loci and 73 showed one mismatch (7.5 %). Mismatches were all ascribed to null alleles, allelic drop-out, or microsatellite stutter problems. The frequency of mismatches was consistent with empirical estimates of genotyping error using repeated genotyping of individuals (Chi-squared test, χ^2=0, df=1, p=0.9986; see above).

Statistical analysis To evaluate the effects of several predictors on probability of EPM occurrence, it was first necessary to control for identity of the attending female (social mother), which was only known for a subset of 225 nests. To do this, we adopted the generalized estimating equation approach (GEE; Zuur et al. 2009) used in *geepack* (Venables and Ripley 2002), as implemented in the R statistical package (R Core Team 2013). This allowed us to model non-normally distributed dependent variables (in the case of IBP and QP, a binary variable [occurrence vs. absence] with a binomial error structure and logit link) as a function of the number of simultaneously breeding females in the breeding area (potential parasites available at the farm), timing of breeding (clutch initiation date, both as linear and second order polynomial), and solitary status of the nest (colonial or solitary nest), while simultaneously controlling for data clustering by female identity (max. 6 observations per individual female). The female identity itself also involves breeding locality (Hamr, Šaloun), because females never switched locality in subsequent years. The correlation structure was set to "exchangeable" (Venables and Ripley 2002). In order to evaluate the effect of parasitism on brood size (apparent or realized), we used the same procedure and applied a Poisson distribution (log link) to the dependent variable (number of young in broods), since residuals from linear models showed non-normal distribution of this variable. These models were run with nest type (nonparasitized, IBP, or QP nest) as an explanatory variable. Full models were backward simplified to achieve the minimum adequate model (see Crawley 2007). Standard statistical tests were used to test for differences in proportion (Sokal and Rohlf 2012). It was possible to estimate the expected proportion of QP young (QPY) resulting by chance (Griffith et al. 2004) using the proportion of EPP offspring (P_{EPP}) detected in our population and the proportion of offspring resulting from EPM (P_{EPM}) as follows: P_{EPP} x $P_{EPM}/(N_{pop})$, where N_{pop} is the number of pairs breeding in the neighborhood (population unit size) available as a source of extra-pair male partners and parasitic females. This prediction provides an upper probability of "chance QP" since it assumes that close neighbors are most likely to parasitize the host.

Results

Frequency of EPM EPM was detected in 54 of 245 nests (22.0 %) between 2010 and 2012, with 60 offspring out of 1060 (5.7 %) identified as EPM (Table S1). Despite the proportion of nests containing EPM young varying from 17.5 % in 2012 to 33.9 % in 2011 (Table S1), there was no significant difference between years in the proportion of EPM young in the population (Chi-squared test, χ^2=4.90, df=2, n=245, p=0.086). While only one parasitic offspring was found in the vast majority of nests, four nests contained two parasitic young (from two different females) and we detected two QPY (both from the same female) in one nest. The identity and nest position of the simultaneously laying nest parasite were known in 18 cases. Interestingly, in all cases, there were other nests available to the parasites that were closer to her nest than the eventual host's nest (range 2–15 nests, mean 6.9 ±0.8 [SE]). The number of EPM offspring detected in nests was unrelated to the number of EPP offspring (Spearman's rank correlation, r_s=−0.051, n=245, p=0.43). Altogether, 65 unhatched eggs were detected in 50 nests, though DNA could only be isolated from four of them. Of these four eggs, none were parasitic and two resulted from extra-pair copulations. The occurrence of unhatched eggs was not associated with occurrence of EPM (unhatched eggs in 42 out of 190 nests without EPM vs. eight out of 56 nests with EPM; Chi-squared test, χ^2=1.63, df=1, p=0.21).

Quasi-parasitism We detected 17 QPY (1.6 % of all 1060 young, 28.3 % of 60 EPM young) in 16 nests (6.5 % of all 245 nests, 29.6 % of 54 nests with EPM; Table S1). QP frequency of occurrence was the same in both the first (14 cases out of 191) and second (3 out of 54) broods (Chi-squared test, χ_1^2= 0.20, p=0.65). EPP was frequent in our population, with 17.2 % of offspring being sired from extra-pair males in 2010, 16.5 % in 2011 and 17.8 % in 2012 (Table S1). Using population-level proportions of EPM and EPP (0.057 and 0.172, respectively), we would expect ~1.0 % QPY for N_{pop}=1, 0.5 % for N_{pop}=2, 0.3 % for N_{pop}=3, 0.2 % for N_{pop}=5, and 0.1 % QPY for N_{pop}=10 if QPY were occurring by chance alone, all figures lower than those actually detected. Moreover, the nest position of the QP female was known in four cases. As with EPM in general, the QP female and host female nests were never in close proximity (maximum distance 120 m). Furthermore, there were 8.25 ($CI_{95\%}$ 6.72–9.77) nests in the same breeding phase (egg-laying period overlapping with the host clutch) closer to the parasitized nest than the QP female's nest, suggesting that N_{pop} was, on average, higher than six in our population. This produced 95 % confidence intervals for "chance QP" in our population of 0.09 to 0.13 % of offspring (similar values were obtained when N_{pop} was calculated based on all EPM cases where the parasite's nest was identified).

Socioecological factors and EPM Using only data from nests where the identity of the attending female was known (N=225 nests from 120 females), we were able to evaluate the effect of social context (solitary or colonial breeding) and time of breeding (polynomial and linear) on probability of EPM occurrence. While our results (see above) indicate that QP is not driven by chance in barn swallows, we pooled that all EPM together as separate tests led to similar results. None of the predictors used had any effect on occurrence of EPM, except for the number of simultaneous egg-laying females (Table 1). We found no regular case of IBP, and only one case of QP, in 21 solitary nests. Note, however, that the effect of nest position was not important in the model where number of simultaneous egg-laying females was included (Table 1). The minimal adequate model (MAM) only contained the latter variable as a sole predictor of EPM probability (Fig. 1) and was highly significant (generalized estimating equation analysis, comparison of null, and MAM models, χ^2=12.8, Δdf=1, p<0.001; slope, 0.14±0.04 [SE]).

EPM and host fitness The apparent brood size (number of 8-day-old nestlings in the nest) for all nests in the population was 4.33±0.07 [SE] (range 1–6, n=245), though there were differences in apparent brood size and realized brood size (i.e., number of offspring of the nest attending female in her nest) between nonparasitized, IBP and QP nests (see Fig. 2). In general, there was a positive relationship between brood size and the number of EPM offspring detected (Spearman's rank correlation, r_s=0.15, n=245, p=0.017). When analysis was restricted to nests with known female identity, apparent brood size increased in nests with IBP and/or QP (GEE model; χ^2=8.46, Δdf=2, p=0.015). Brood size estimated from the model with female identity included as a random effect was 4.21 young ($CI_{95\%}$ 4.05–4.37) for nonparasitized nests, 4.67 ($CI_{95\%}$ 4.63–4.71) for nests with IBP and 4.66 ($CI_{95\%}$ 4.62–4.70) for QP nests. Hence, brood size was exactly the same in IBP and QP nests. Note that QP increases reproductive output of the nest-attending male above the population average as he sires the parasitic offspring. On the other hand, realized brood

Table 1 Generalized estimating equation analysis (GEE; for details, see the Methods section) of the probability of a barn swallow nest being parasitized as a function of selected socioecological factors

	Estimate	SE	Wald statistics	P
Intercept	−2.15	0.36	34.28	<0.001
Timing (linear)	−0.808	2.738	0.09	0.768
Timing (polynomial)	2.618	2.497	1.10	0.294
Solitary/colonial	−1.658	1.032	2.58	0.108
Parasites available	0.135	0.039	11.68	<0.001

Intraspecific brood parasitism and quasi-parasitism events were combined. Only nests with known female identity were used (n=225 nests of 120 females; female identity as a random effect). Julian date of clutch initiation (timing) is included as either a linear or second-order polynomial term; and solitary breeding (1) or colony breeding (0) included as a factor (solitary/colonial). The number of available parasites was calculated per nest and per each locality as the number of females with at least partly overlapping clutch laying as the focal nest. The full model presented here is highly significant (χ^2 =19.2, ΔDf=4, p<0.001)

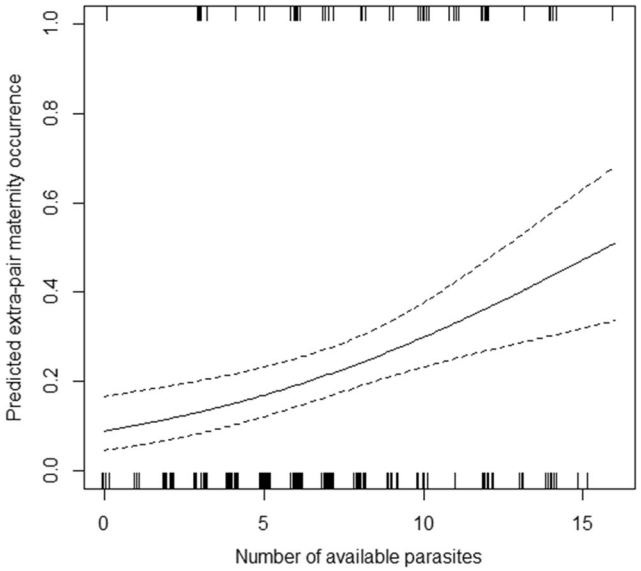

Fig. 1 Predicted proportion of parasitised nests (occurrence of extra-pair maternity) as a function of the number of potentially available parasites (number of females laying eggs simultaneously as the focal female) available at the locality (farm). The figure is based on a logistic regression model of 245 nests for which information about extra-pair maternity occurrence was available. *Dashed lines* represent 95 % CIs

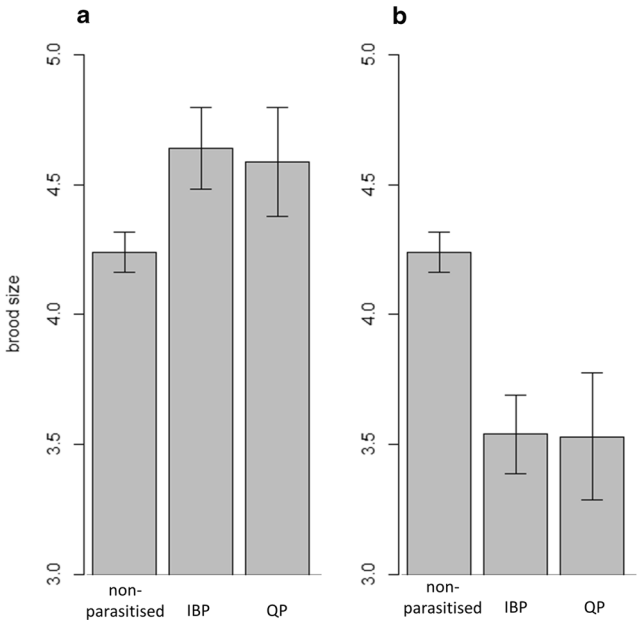

Fig. 2 Mean apparent (**a**) and realized (from the host female point of view; **b** brood size in barn swallows). *Vertical bars* denote $CI_{95\%}$ intervals around mean values. Nonparasitized—nests with no extra-pair maternity detected; IBP—nests with regular intraspecific brood parasitism; QP—nests with quasi-parasitism. Data based on 245 nests from South Bohemia (Doudlebia, Czech Republic) examined between 2010 and 2012

size for the host female was reduced in nests suffering from IBP or QP, with the effect of nest status (IBP, QP, or nonparasitized) being highly influential (GEE model; χ^2= 13.8, Δdf=2, p=0.001). Mean estimated realized brood size for the host female was 3.56 in IBP nests ($CI_{95\%}$ 3.52–3.60) and 3.60 in QP nests ($CI_{95\%}$ 3.55–3.65).

Parasite fitness At least one EPM nestling was found in four (22.0 %) out of 18 simultaneously laying brood parasite nests, the same value as that for the overall proportion of broods with EPM detected in a population (Table S1). The parasitic strategy resulted in an average of 5.33±0.17 [SE] (range 4–7; $CI_{95\%}$ 4.95–5.71, N=18 females) own offspring produced by the parasitic female per breeding attempt (a combination of offspring found in her own and host's nest) which is higher than that estimated for nonparasitized nests in the population (see above).

Discussion

Intraspecific brood parasitism appears to be particularly common in avian lineages with precocial modes of parental care (ratites and Galloanserae) but has also been detected in various altricial species (e.g., Yom-Tov 2001). In this study, we used molecular methods to document the occurrence of EPM (both regular brood parasitism [IBP] and QP) in a population of small, socially monogamous barn swallows. Though the barn swallow is a model species in the field of behavioral ecology (e.g., Møller 1994), and many studies have focused on molecular determination of parentage in barn swallow populations across the globe (e.g., Møller et al. 2003; Safran et al. 2005; Laskemoen et al. 2013; Vortman et al. 2013), regular brood parasitism has not yet been detected in this species using molecular methods. However, brood parasitism has been suggested for one (Danish) barn swallow population using traditional parasitic event detection methods (e.g., more than one egg laid in a clutch per day) with 16.5 % nests containing EPM eggs (Møller 1987). Our estimate is slightly higher, with 22.0 % of nests displaying EPM. It is difficult to explain the relatively high levels of EPM detected in our population, especially compared to other studies that have used molecular methods to detect EPM. One potential explanation may be related to the large colonies studied in our case, with high population densities encouraging high parasite availability. It is already known that IBP increases with population density (e.g., see Bennett and Owens 2002). Another possibility is that barn swallow subspecies differ in EPM levels, which would explain differences between our population and at least two others (Safran et al. 2005; Vortman et al. 2013).

It should be noted that our EPM values could still be slight underestimates since we sampled 8-day-old nestlings, meaning that some parasitic events could have remained undetected if, for example, barn swallows utilize efficient antiparasitic strategies such as parasitic egg rejection. This appears not to have been the case, however, as conspecific egg rejection after clutch initiation has never been observed in European barn swallows (see Møller 1987) and even rejection of interspecific model cuckoo eggs is absent (reviewed in Liang et al. 2013). Parasitic nestlings could also experience increased mortality compared to the rest of the brood if parents recognize and discriminate against parasitic nestlings in the nest. Partial brood mortality prior to blood sampling (DNA) was low in our population (only two nestlings), however, though it should be noted that we were unable to estimate maternity for 61 unhatched eggs. While it appears that unhatched eggs did not result primarily from EPM (none of the four eggs for which DNA was extracted were EPM), we cannot exclude the possibility that the proportion of EPM eggs in this subsample was higher than that indicated by the levels observed in the population.

Our results indicated that one ~30 % of all EPM nestlings were actually sired by the male attending the nest. Such QP has so far been reported for just a few avian species (e.g., Wrege and Emlen 1987; Griffith et al. 2004; Krakauer 2008; Li et al. 2008; Berger et al. 2013). While the overall proportion of QPY in our population was only ~2 %, it still requires an explanation. There are two possibilities; either QP occurs by chance, resulting from a combination of two reproductive strategies (EPP and IBP), or it is an adaptive behavior benefiting the local male, the parasitic female, or both (see Griffith et al. 2004; Berger et al. 2013). In our case, it was possible to calculate the probability that QP would occur by chance, given the known proportions of EPM and EPP in our population. It appears that breeding population units (neighbors [N *sensu* Griffith et al. 2004]) would need to be very small (~1 neighbor) to result in ~1 % of QPY by chance. In fact, this calculation is conservative as it assumes that close neighbors (members of the breeding unit) are the most likely brood parasites, as suggested in one previous study based on indirect observations of egg-color patterns (Møller 1987). Our results, however, indicate that parasitism in barn swallows could take place on a larger spatial scale. Of the 28 cases where parasitic female nest position was identified in our study, the host's direct neighbor was the source in only one case. As with all EPM, identified QP females were never the closest neighbor to the parasitized nests, again suggesting a larger spatial scale within which egg dumping takes place. These results strongly suggest, therefore, that the QP observed in our population did not occur by chance, sensu Alves and Bryant (1998). QP has been observed in one other barn swallow population, though, surprisingly, no IBP was detected in this population (Møller et al. 2003). Further, some nests contained up to three QPY, and it remains unclear whether these cases resulted from rapid mate switching or misidentification (Griffith et al. 2004). In our case, we can rule out the possibility that QP females were

previous social partners of the nest attending males or that QP resulted from nest takeover, with a relatively high certainty. First, QP occurred with the same frequency among first and second broods. In second broods, it occurred in pairs followed from the beginning of the breeding season where both partners were identified based on unique combination of color rings. Hence, mate switching is an unlikely explanation for the occurrence of QP in second breeding attempts of the same pair. In a follow-up study (AP and TA, unpublished data), we have also identified five QP offspring in five first-breeding clutches where both nest owners (pair members) were color banded prior to the egg-laying and no mate switching occurred. Furthermore, if QP was the result of mate switching, one would predict that these eggs would be laid at the beginning of the laying sequence. In cases that have been observed in detail (n=4), however, QP eggs were laid in the middle of the egg-laying sequence, or as the last egg in the clutch (AP and TA, unpublished data). Unlike Møller et al. (2003), only one QPY was usually found in a nest, which is similar to the situation observed with regular IBP.

As with other alternative reproductive strategies, such as EPP (Griffith et al. 2002), both intraspecific and interspecific variation in parasitism rate appear to be heavily affected by population density (Bennett and Owens 2002). As has been documented in other brood parasitism systems (Rohwer and Freeman 1989; Lyon 1993; Moskát et al. 2006), high breeding synchrony between the host and parasite may increase parasitism rates as more host nests and parasitic females are available in the population at the same time (McRae 1997). Indeed, in our population, the probability of a nest being parasitized strongly depended on the current number of simultaneously egg-laying females. This, in combination with the analysis of parasite/host nest position (see above), indicates that parasite/host interactions in barn swallow nests take place at the breeding colony level. Following the same logic, solitary nests should be less prone to EPM. Indeed, in our study population, females nesting outside the colony tended to be less parasitized than females nesting inside the colony, though the difference was not statistically significant in models including the number of simultaneously laying females at the site (see below).

We found no evidence that timing of breeding per se affected the probability of a nest suffering from EPM. In many precocial species, nesting parasites (parasitic females with their own clutch) or floater females tend to lay parasitic eggs at the beginning of the breeding season (Sorenson 1991; Lyon 1993; Åhlund and Andersson 2001); however, parasitic eggs may also be laid later in the season by young and/or weak females (Spurr and Milne 1976; Baillie and Milne 1982; Laurila and Hario 1988) or by those that have experienced brood failure (Eadie et al. 1988). In our population, some of these strategies could have combined to result in the lack of association between EPM and breeding date. To address this, further research should focus on identification of parasitic females in the population and track their individual nesting histories.

We were able to identify nests of parasitic females in only a limited number of cases. However, these females on average produced higher number of offspring than was the population average. Our data thus indicate that parasitic strategy could be strongly beneficial for breeding barn swallow females (also see Kendra et al. 1988; Åhlund and Andersson 2001). Questions remain as to whether EPM (IBP and QP) is costly for the host female. If this were the case, one would expect evolution of antiparasitic strategies such as egg rejection, nest abandonment, or nest guarding (Lyon and Eadie 2008). Indeed, a previous observational study has indicated a reduction in host clutch size in response to brood parasitism (Møller 1987), while we observed a significant negative impact of brood parasitism on the fitness of the nest attending pair (QP is only harmful to the attending female). While apparent brood size was significantly enlarged in the host nest, possibly resulting in higher parental investment and/or lowered nestling survival (Boonekamp et al. 2014), the actual number of offspring genetically related to the host female was reduced compared to the population average. Several nonmutually exclusive scenarios could help explain reduced brood size in the host nest. First, the host female may reduce her own egg production, perhaps because a clutch with a parasitic egg evokes a completed clutch (Andersson and Eriksson 1982; Erikstad and Bustnes 1994). Second, the parasitic female could remove one of the host's eggs prior the parasitic event, as documented for cliff swallow (Brown and Brown 1988) and other passerines (e.g., Lombardo et al. 1989; Hoi et al. 2010). Parasitic females may also actively choose hosts with small clutches to minimize competition between the parasitic and host nestlings (Brown 1984), or choose a host of inferior condition in order to simplify access to the host nest (Møller 1987). Based on our data, we cannot fully distinguish between these scenarios. Despite potential fitness costs associated with the appearance of a parasitic egg in the clutch (discussed in Lyon et al. 2002, and also suggested by our data), the average brood size for both IBP and QP nests was larger than the population average, and there was a significant positive correlation between the brood size recorded and the number of parasitic (IBP or QP) offspring in it. Collectively, this indicates that there is no one-for-one replacement of host offspring by parasitic chicks (or that $C<1$ sensu Lyon et al. 2002) in barn swallows. Interestingly, our results thus do indicate that, in contrast to their social partners, barn swallow males may increase the number of offspring reared by engaging in QP and cooperating with parasitic females, because brood sizes were higher in QP nests compared to nonparasitized nests in the population, though we cannot exclude the possibility that compensatory mortality of offspring occurs later in the year (e.g., Lyon et al. 2002). There are at least two scenarios worth

of evaluating: (1) brood size may be limited by egg-laying capacity of females in barn swallows, not post laying parental care, so males can increase fitness by having a second female add eggs to the clutch (as long as he sires them), or (2) the extra eggs added to the clutch by the QP female make the host female work harder as a parent, not the male. The occurrence of QP in our population remains a mystery and represents fruitful area for future research. Since both male and female swallows guard the nest against brood parasites during egg-laying (Møller 1994), further research is needed to evaluate potential conflicts that may arise between nest-attending males and their mates over access of egg-laying parasitic females.

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Authorship The project was designed by TA, JC, RM, and AP; data was collected by all authors; paternity and maternity analysis was conducted by JK, RM, and PM; TA and AP analyzed the data for this paper; TA, RM, and AP wrote the manuscript with contributions from all coauthors.

Ethical standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All protocols were noninvasive and adhered to the laws and guidelines of the Czech Republic (Czech Research Permit numbers 6628/2008-10001). All protocols were approved by the Animal Care and Use Committees at the Czech Academy of Sciences (041/2011), and Charles University in Prague (4789/2008-30).

References

Åhlund M, Andersson M (2001) Female ducks can double their reproduction. Nature 414:600–601

Alves MAS, Bryant DM (1998) Brood parasitism in the sand martin, *Riparia riparia*: evidence for two parasitic strategies in a colonial passerine. Anim Behav 56:1323–1331

Amos W, Hoffman JI, Frodsham A, Zhang L, Best S, Hill AVS (2007) Automated binning of microsatellite alleles: problems and solutions. Mol Ecol Notes 7:10–14

Andersson M (1984) Brood parasitism within species. In: Barnard CJ (ed) Producers and scroungers. Croom Helm, London, pp 195–228

Andersson M, Åhlund M (2001) Protein fingerprinting: a new technique reveals extensive conspecific brood parasitism. Ecology 82:1433–1442

Andersson M, Eriksson MOG (1982) Nest parasitism in Goldeneyes *Bucephala clangula*: some evolutionary aspects. Am Nat 120:1–16

Ar A, Yom-Tov Y (1978) The evolution of parental care in birds. Evolution 32:655–669

Arnold KE, Owens IPF (2002) Extra-pair paternity and egg dumping in birds: life history, parental care and the risk of retaliation. Proc R Soc Lond B 269:1263–1269

Baillie SR, Milne H (1982) The influence of female age on breeding in the Eider *Somateria mollissima*. Bird Study 29:55–66

Bennett PM, Owens IPF (2002) Evolutionary ecology of birds. Oxford University Press, Oxford

Berger I, Dvir Y, Leshem Y, Yom-Tov Y, Markman S (2013) Extra-pair copulations, intra-specific brood parasitism, and quasi-parasitism in birds: a theoretical approach. Acta Ethol 17:131–140

Boonekamp JJ, Salomons M, Bouwhuis S, Dijkstra C, Verhulst S (2014) Reproductive effort accelerates actuarial senescence in wild birds: an experimental study. Ecol Lett 17:599–605

Brown CR (1984) Laying eggs in a neighbor's nest: benefit and cost of colonial nesting in swallows. Science 224:518–519

Brown CR, Brown MB (1988) The costs and benefits of egg destruction by conspecifics in colonial cliff swallows. Auk 105:737–748

Brown CR, Brown MB (1989) Behavioural dynamics of intraspecific brood parasitism in colonial cliff swallows. Anim Behav 37:777–796

Brown CR, Brown MB (1991) Selection of high-quality host nests by parasitic cliff swallows. Anim Behav 41:457–465

Crawley MJ (2007) The R Book. Wiley, West Sussex

Dawkins R (1980) Good strategy or evolutionarily stable strategy. In: Barlow GW, Silverberg J (eds) Sociobiology: beyond nature/nurture? Westview Press, Boulder, pp 331–367

Eadie JM (1989) Alternative female reproductive tactics in a precocial bird: The ecology and evolution of brood parasitism in goldeneyes. PhD Thesis, University of British Columbia, Vancouver

Eadie JM, Kehoe FP, Nudds TD (1988) Pre-hatch and post-hatch brood amalgamation in North American Anatidae: a review of hypotheses. Can J Zool 66:1709–1721

Emlen ST, Wrege PH (1986) Forced copulations and intra-specific parasitism: two costs of social living in the White-fronted Bee-eater. Ethology 71:2–29

Erikstad KE, Bustnes JO (1994) Clutch size determination in common eiders: an egg removal and egg addition experiment. J Avian Biol 25:215–218

Fujita G, Higuchi H (2007) Barn swallows prefer to nest at sites hidden from neighboring nests within a loose colony. J Ethol 25:117–123

Gibbons DW (1986) Brood parasitism and cooperative nesting in the moorhen, *Gallinula-chloropus*. Behav Ecol Sociobiol 19:221–232

Griffith SC, Owens IPF, Thuman KA (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. Mol Ecol 11:2195–2212

Griffith SC, Lyon B, Montgomerie R (2004) Quasi-parasitism in birds. Behav Ecol Sociobiol 56:191–200

Griffith SC, Barr I, Sheldon BC, Rowe LV, Burke T (2009) Egg patterning is not a reliable indicator of intraspecific brood parasitism in the blue tit *Cyanistes caeruleus*. J Avian Biol 40:337–341

Hoi H, Darolová A, Krištofík J (2010) Conspecific brood parasitism and anti-parasite strategies in relation to breeding density in female bearded tits. Behaviour 147:1533–1549

Jackson WM (1993) Causes of conspecific nest parasitism in the northern masked weaver. Behav Ecol Sociobiol 32:119–126

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099–1106

Kendra PE, Roth RR, Tallamy DW (1988) Conspecific brood parasitism in the house sparrow. Wilson Bull 100:80–90

Kleven O, Jacobsen F, Robertson RJ, Lifjeld JT (2005) Extrapair mating between relatives in the barn swallow: a role for kin selection? Biol Lett 1:389–392

Krakauer AH (2008) Sexual selection and the genetic mating system of wild turkeys. Condor 110:1–12

Laskemoen T, Albrecht T, Bonisoli-Alquati A, Cepák J, de Lope F et al (2013) Variation in sperm morphometry and sperm competition among barn swallow (*Hirundo rustica*) populations. Behav Ecol Sociobiol 67:301–309

Laurila T, Hario M (1988) Environmental and genetic factors influencing clutch size, egg volume, date of laying and female weight in the common eider *Somateria mollissima*. Finn Game Res 45:19–30

Li MH, Välimäki K, Piha M, Pakkala T, Merilä J (2008) Extrapair paternity and maternity in the Three-toed Woodpecker, *Picoides tridactylus*: insights from microsatellite-based parentage analysis. PLoS ONE 4, e7895

Liang W, Yang CC, Wang LW, Møller AP (2013) Avoiding parasitism by breeding indoors: cuckoo parasitism of hirundines and rejection of eggs. Behav Ecol Sociobiol 67:913–918

Lombardo MP, Power HW, Stouffer PC, Romagnano LC, Hoffenberg AS (1989) Egg removal and intraspecific brood parasitism in the European starling (*Sturnus vulgaris*). Behav Ecol Sociobiol 24: 217–223

Lyon BE (1993) Conspecific brood parasitism as a flexible female reproductive tactic in American coots. Anim Behav 46:911–928

Lyon BE, Eadie JM (2008) Conspecific brood parasitism in birds: a life-history perspective. Annu Rev Ecol Evol Syst 39:343–363

Lyon BE, Everding S (1996) High frequency of conspecific brood parasitism in a colonial waterbird, the Eared Grebe *Podiceps nigricollis*. J Avian Biol 27:238–244

Lyon BE, Hochachka WM, Eadie JM (2002) Paternity-parasitism trade-offs: a model and test of host-parasite cooperation in an avian conspecific brood parasite. Evolution 56:1253–1256

MacWhirter RB (1989) Minireview: on the rarity of intraspecific brood parasitism. Condor 91:485–492

McRae SB (1997) A rise in nest predation enhances the frequency of intraspecific brood parasitism in a moorhen population. J Anim Ecol 66:143–153

Møller AP (1987) Intraspecific nest parasitism and anti-parasite behaviour in swallows, *Hirundo rustica*. Anim Behav 35:247–254

Møller AP (1994) Sexual selection and the barn swallow. Oxford University Press, Oxford

Møller AP, Brohede J, Cuervo JJ, de Lope F, Primmer C (2003) Extrapair paternity in relation to sexual ornamentation, arrival date, and condition in a migratory bird. Behav Ecol 14:707–712

Moskát C, Barta Z, Hauber ME, Honza M (2006) High synchrony of egg laying in common cuckoos (*Cuculus canorus*) and their great reed warbler (*Acrocephalus arundinaceus*) hosts. Ethol Ecol Evol 18: 159–167

Nielsen CR, Parker PG, Gates RJ (2006) Intraspecific nest parasitism of cavity-nesting wood ducks: costs and benefits to hosts and parasites. Anim Behav 72:917–926

Payne RB (1977) The ecology of brood parasitism in birds. Annu Rev Ecol Syst 8:1–28

Primmer CR, Møller AP, Ellegren H (1995) Resolving genetic relationships with microsatellite markers: a parentage testing system for the swallow *Hirundo rustica*. Mol Ecol 4:493–498

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rohwer FC, Freeman S (1989) The distribution of conspecific nest parasitism in birds. Can J Zool 67:239–253

Safran RJ, Neuman CR, McGraw KJ, Lovette IJ (2005) Dynamic paternity allocation as a function of male plumage color in barn swallows. Science 309:2210–2212

Saino N, Primmer CR, Ellegren H, Møller AP (1997) An experimental study of paternity and tail ornamentation in the barn swallow (*Hirundo rustica*). Evolution 51:562–570

Sayler RD (1992) Ecology and evolution of brood parasitism in waterfowl. In: Batt BDJ, Afton AD, Anderson MG, Ankney CD, Johnson DH, Kadlec JA, Krapu GL (eds) Ecology and management of breeding waterfowl. University of Minnesota Press, Minneapolis, pp 290–322

Semel B, Sherman PW (2001) Intraspecific parasitism and nest-site competition in wood ducks. Anim Behav 61:787–803

Sokal RR, Rohlf FJ (2012) Biometry. W. H. Freeman & Co, New York

Sorenson MD (1991) The functional significance of parasitic egg laying and typical nesting in redhead ducks: an analysis of individual behaviour. Anim Behav 42:771–796

Sorenson MD (1992) Comment: why is conspecific nest parasitism more frequent in waterfowl than in other birds? Can J Zool 70:1856–1858

Spurr EB, Milne H (1976) Factors affecting laying date in the Common Eider. Wildfowl 27:107–110

Svensson L (1984) Identification guide to European passerines, 2nd edn. Svensson, Stockholm

Tsyusko OV, Peters MB, Hgen C, Tuberville TD, Mousseau TA, Møller AP, Glenn TC (2007) Microsatellite markers isolated from barn swallows (*Hirundo rustica*). Mol Ecol Notes 7:833–835

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Venables WN, Ripley BD (2002) Modern applied statistics with S. Springer, New York

Vortman Y, Lotem A, Dor R, Lovette I, Safran R (2013) Multiple sexual signals and behavioral reproductive isolation in a diverging population. Am Nat 182:514–523

Waldeck P, Öst M, Kilpi M, Andersson M (2004) Brood parasitism in a population of common eider (*Somateria mollissima*). Behaviour 141:725–739

Wang JL (2004) Sibship reconstruction from genetic data with typing errors. Genetics 166:1963–1979

Wrege PH, Emlen ST (1987) Biochemical determination of parental uncertainty in white-fronted bee-eaters. Behav Ecol Sociobiol 20:153–160

Yom-Tov Y (1980) Intraspecific nest parasitism in birds. Biol Rev 55:93–108

Yom-Tov Y (2001) An updated list and some comments on the occurrence of intraspecific nest parasitism in birds. Ibis 143:133–143

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York

Online supplementary material (Tables S1, S2, S3) for *Behavioral Ecology and Sociobiology*

Title: Brood parasitism and quasi-parasitism in the European Barn Swallow *Hirundo rustica rustica*

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Online material Table S1 Prevalence of intraspecific parasitism, quasi-parasitism and extrapair paternity (EPP) in a South Bohemian (Doudlebia, Czech Republic, localities Hamr and Saloun) barn swallow population. EPM young numbers combine parasitic and quasiparasitic young.

Year	Nests	Parasitic nests (%)	Quasi-parasitic nests (%)	Young	EPM young (%)	EPP nests (%)
2010	66	9 (13.6)	4 (6.1)	269	14 (5.2)	30 (45.4)
2011	59	15 (25.4)	5 (8.5)	279	23 (8.3)	27 (45.7)
2012	120	14 (11.7)	7 (5.8)	512	23 (4.5)	47 (39.2)
Total:	245	38 (15.5)	16 (6.5)	1060	60 (5.7)	104 (42.4)
Hamr	100	10 (10.0)	7 (7.0)	426	18 (4.2)	37 (37.0)
Saloun	145	28 (19.0)	9 (6.0)	634	42 (6.6)	67 (46.0)

Online material Table S2 Summary statistics for microsatellite loci used to determine conspecific brood parasitism in barn swallows. Locus – locus name as given in Primmer et al. 1996 or Tsyusko et al. 2007; N – number of genotyped individuals; K – number of alleles; H(obs) – observed heterozygosity; H(exp) – expected heterozygosity; PIC – polymorphic information content; F(Null) – frequency of null alleles, labelling – fluorescent dye used for forward primer labelling; concentration - concentration (μM) of primers in PCR reactions. Equal concentrations of forward and reverse primer were used.

Locus	K	N	H(obs)	H(exp)	PIC	F(Null)	labelling	concentration
Hir15	12	1663	0.652	0.682	0.627	0.0230	6-FAM	0.15
Hir10	13	1664	0.788	0.835	0.815	0.0280	HEX	0.15
Hir20	18	1664	0.826	0.843	0.824	0.0090	HEX	0.5
Hir6	16	1662	0.857	0.842	0.822	-0.0092	NED	0.05
Hir22	19	1663	0.829	0.876	0.863	0.0263	NED	0.05
HrU10	47	1661	0.950	0.952	0.948	0.0464	PET	0.5

Online material Table S3 Occurrence of extra-pair maternity (EPM) and extra pair paternity (EPP). Nests are classified according to known/unknown status of social parents.

	Both social parents known	Only female known	Only male known	Unknown social parents
Total nests	217	8	13	7
Nests with EPM	50	2	0	3
Nests with EPP	91	5	8	6

PUBLIKACE 2

Tomášek O., Pap P. L., Adámková M., Cepák J., Fulop A., **Michálková R.**, Stermin A. N., Vágási C. I., Vincze O. & Albrecht T. **Age-dependence and viability signaling function of tail streamer length in the European barn swallow.** (*In preparation for Journal of Animal Ecology*)

Foto: Vladimír Pokorný

Age-dependence and viability signalling function of tail streamer length in the European barn swallow

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Abstract

1. Sexual ornaments are often assumed to evolve as signals of individual viability. Ornaments can also signal individual age, thereby advertising proven viability. Although age-related increase in ornament expression has often been reported at the population level, only a few studies have separated the within-individual phenotypic change from selective disappearance due to differential viability of individuals with distinct phenotypes. Even less such studies analysed senescence in ornament expression.
2. In this study, we tested viability signalling function and senescence of tail streamer length in the European barn swallow (*Hirundo rustica rustica*) in two populations in the Czech Republic and Romania. In addition, we experimentally manipulated tail streamer length and analysed survival and tail length in the following year to test for tail streamer costs.
3. Using linear mixed-effect modelling (LMM) approach separating within- and between-individual age-related effects, we found non-linear within-individual increase in tail length with age in both populations and sexes, with highest increase between the first and the second year of adult life and subsequent levelling-off. There was no evidence for senescence in this ornamental trait in any of the population or sex.
4. Contrary to some previous studies on European barn swallows, the LMM approach showed a positive correlation between tail length and lifespan in males, but not females, across both populations. In addition, using logistic survival modelling, we found that tail length in the first year of life predicts lifelong survival in a non-linear way in Czech, but not Romanian, males. In the Czech population, males having tail streamers 5 mm longer than population median were the best survivors.
5. Experimental manipulations of tail streamer length had no significant effect on either survival or tail length in the following year.

6. Our data suggest that tail streamer length can signal both proven (age) and potential viability in the European barn swallows, though between-population differences in signalling content may exist. We also found that the directional selection for longer tail streamers may be combined with stabilising selection acting on males with extremely long tails. We further suggest that previous reports of senescence in tail length in this subspecies may be false positive and may have resulted from using inappropriate approaches. Interestingly, our data suggest that costs of tail streamers are at best mild, and only associated with extremely long tails.

Introduction

The indicator mechanism models of sexual selection assume that sexually selected ornamental traits are expressed in a condition-dependent manner and as such can honestly signal individual quality (Zahavi 1975; Grafen 1990; Getty 1998; Hill 2011). In such a case, sexual signals are expected to show positive relationship between their expression and survival (Jennions *et al.* 2001). Nonetheless, direction of the correlation could change due to a trade-off between ornamentation and survival as sexual signals are usually assumed to be costly (Zahavi 1975). The resultant direction of the correlation between two traits that are traded off is then influenced by resource acquisition (Van Noordwijk & de Jong 1986). In contrast, Fisherian runaway process does not incorporate condition-dependence and predict negative correlation between sexual character expression and survival (Lande 1981; Brooks 2000). It has been suggested, however, that the pure Fisherian process and the indicator mechanism are two ends of the sexual selection continuum and that the direction of correlation between ornamentation and survival may depend on the costliness of the female choice, and thus the intensity of sexual selection that females impose on males (Kokko *et al.* 2002). Hence, negative or no correlation between male attractiveness and survival indicates low costs of female choice imposing strong sexual selection on males, whereas positive correlation indicates more relaxed sexual selection due to high female choice costliness, which allows secondary sexual characters to function as viability indicators (Kokko *et al.* 2002).

Sexual traits can also advertise proven viability through signalling individual's age (Manning 1985). Game theoretic modelling has shown that such an increasing investment in attractiveness with age is in accordance with life-history theory (Kokko 1997, 1998). Although, many empirical studies have analysed age-related changes in male ornamental traits, most of them did not control for selective disappearance of particular phenotypes. Results of such studies, even those using longitudinal data, cannot be interpreted as within-individual

phenotypic change with age, because statistical separation of between-individual variation in lifespan is necessary to obtain unbiased estimates of age-related changes within individuals (van de Pol & Verhulst 2006; Nussey *et al.* 2008). The few studies that used such statistical decomposition of within- and between-individual effects usually reported increasing pre-senescent expression of secondary sexual characters with age (Delhey & Kempenaers 2006; Bitton & Dawson 2008; Nussey *et al.* 2009; Val *et al.* 2010; Balbontín *et al.* 2011; Evans *et al.* 2011; Kervinen *et al.* 2015), except for one recent study reporting no age-related change in pre-senescent carotenoid-based beak colouration in male zebra finches (Simons *et al.* 2016).

Moreover, only a proportion of these studies provided an analysis of senescence in sexual display, most probably due to the difficulty of obtaining necessary longitudinal data (Evans *et al.* 2011). The few studies available to date reported mixed results, with senescence in secondary sexual characters being detected is some studies (Balbontín *et al.* 2011; Kervinen *et al.* 2015; Simons *et al.* 2016) but not in others (Nussey *et al.* 2009; Evans *et al.* 2011). Furthermore, in some of the studies reporting senescence in male ornamental traits, such a conclusion is only based on a significant quadratic age effect (e.g. Balbontín *et al.* 2011). The information content of significant quadratic age effect as an evidence for senescence is limited, however, as significant quadratic age effect may solely result from the increase in trait expression being steeper in early life with subsequent slowdown or levelling-off (Bouwhuis *et al.* 2009). Overall, our current knowledge on how within- and between-individual effects determine the observed patterns of age-related expression of secondary sexual characters at the population level appears to be only fragmentary, and more longitudinal studies separating age effects at different levels and properly testing for senescence are needed to clarify this issue (Simons *et al.* 2016).

The evolution of condition-dependent sexual signals implies an existence of mechanisms maintaining signalling honesty. Handicap principle of signalling honesty maintenance assumes

that the expression of sexual signals is costly and that the costs are higher for low quality individuals. Therefore, only individuals of high quality can express elaborate signals without reducing their overall fitness (Zahavi 1975; Grafen 1990). Recently, several alternative hypotheses have been put forward, proposing that signal expression may depend on metabolic and signalling pathways that are fundamental for organism functionality and performance, thereby signalling individual quality and condition in a cost-free manner (Hill 2011; Emlen *et al.* 2012; Warren *et al.* 2013). Testing costs associated with ornament elaboration can therefore provide a clue to the general mechanism maintaining signalling honesty.

In this study, we use longitudinal data from the two long-term studied populations of the European barn swallow (*Hirundo rustica rustica*) from the Czech Republic (CZ) and Romania (RO) to test whether expression of a sexually selected trait, the length of tail streamers, reflects individual age and survival and to estimate the effect of ageing on this trait. Tail streamers of barn swallow males are much longer than those of females and rank among the most iconic examples of sexual selection (Møller 1994b). We use linear mixed-effect models (LMM) fitted with both individual age and lifespan as predictors of tail length to decompose within- and between-individual effects of age on tail length expression in both sexes (van de Pol & Verhulst 2006). We also tested whether tail length in the first year of adult life predicts lifelong survival using logistic discrete-time hazard models with logit link-function, death set as a binary dependent variable and tail length in the first year of adult life fitted among the predictors (Singer & Willett 2003).

In addition, we performed an experimental manipulation of male tail length in the CZ population in order to test the costs of tail streamers. Specifically, we tested whether manipulation of tail streamer length affects survival to the next year or tail streamer growth during subsequent moult. The experimental manipulation is the best means to evaluate ornamentation costs, because, in observational studies, the costliness may be confounded by

variability in individual genetic quality (Grafen 1990) and/or differences in resource availability (Van Noordwijk & de Jong 1986).

Methods

Study sites and data collection

We collected longitudinal data from two populations of the European barn swallow in the Czech Republic and Romania. The CZ population has been studied at four isolated farms in the Třeboňsko Protected Landscape Area: Hamr in Lužnice (49°03'24"N, 14°46'10"E); Šaloun in Lomnice nad Lužnicí (49°04'08"N, 14°42'39"E); Obora in Třeboň (48°59'07"N, 14°46'50"E); and Břilice (49°01'14"N, 14°44'17"E); during the breeding seasons 2010–2017. In this population, most of the birds breed in colonies in stables, with just a few solitary pairs breeding in separate rooms or buildings. The RO population has been studied in Cojocna village in central Transylvania (46°45'N, 23°50′E) during the breeding seasons 2011–2017. At this study site, barn swallows usually breed in stall buildings in natural nests most often solitarily, but in some cases several pairs aggregate into small loose colonies (for details see Fülöp *et al.* 2017). In total, we collected 621 observations from 398 CZ males, 469 observations from 293 CZ females, 237 observations from 160 RO males and 283 observations from 179 RO females.

Adult individuals were systematically captured with mist nets or nest traps on several occasions during the breeding season. Upon capture, each individual was marked with a unique aluminium ring and tail streamer length was measured to the nearest mm. Previous observations on barn swallows (Saino *et al.* 1999; Schaub & Von Hirschheydt 2009) and our own capture–recapture data (Pap *et al.* 2005) indicate high breeding site fidelity. Given the extremely high breeding site fidelity and because we captured and marked the vast majority of adult individuals at our study sites every year, unmarked adult birds were assumed to be one-year old birds immigrating from other colonies and birds that did not return to the study site in the next year were regarded as dead (Møller & De Lope 1999; Saino *et al.* 1999; Pap *et al.* 2005; Balbontín

et al. 2011; Costanzo *et al.* 2017). In the CZ population, only 2.1% of adult birds that were captured at least in two years ($N = 243$) were missed in one year between captures. Sex was determined by visual examination of the presence of a brood patch, which develops only in females. In the CZ population, feather samples of at least 10 feathers were collected from the ventral region of the body for subsequent colour analysis.

Ornament manipulations

To assess costs of tail streamers and ventral colouration, we performed experimental manipulation of their expression in the CZ population during breeding seasons 2011–2015. The manipulation experiment was carried out between the first and the second nesting attempt. Tail streamers were elongated or shortened by two standard deviations (15 mm). We cut the streamers 15 mm from the base and, to the stump, we attached new streamers obtained from another male either during the same field session or during one of the previous sessions. The attachment was done by insertion of the entomological pin (total length ca. 10 mm) inside the shaft of the stump and the new streamer was slid onto the protruding half of the pin. The joint was fixed with cyanoacrylate superglue (Loctite, Henkel ČR, Czech Republic). This method preserve the natural proportions of tail streamers and prove successful in the previous experiments (Bro-Jørgensen *et al.* 2007; Vortman *et al.* 2013; Safran *et al.* 2016).

In addition, tail length manipulation was combined with experimental darkening of ventral colouration in 2014 and 2015, to evaluate its costs and importance for reproductive success. All the feathers of ventral region were darkened using a non-toxic permanent marker (Prismacolor, light walnut) following previous experimental studies on barn swallows (Vortman *et al.* 2013; Safran *et al.* 2016).

Plumage colouration measurement

Ten feathers were composed on each other and fixed on a white paper index cards to achieve layer equivalent to real ordering of feathers in birds (Quesada & Senar 2006). Feather reflectance was measured between 300–700 using AvaSpec 2048 spectrometer with an AvaLight-XE light source (Avantes, Netherlands). Custom-made adapter was attached on the sensing spectrometer probe to eliminate ambient light and ensure constant distance 3.5 mm between the probe and the sample. Each sample was measured three times at the distal part of the feathers with the probe held perpendicular to the feather surface. Spectrometer was calibrated against a darkroom and a white standard (WS-2, Avantes, Netherlands) after measuring every eight samples. Data were analysed using R 3.4.3 (R Core Team 2017) and pavo 1.3.1 R package (Maia *et al.* 2013). Three measurements of each sample were averaged and smoothed (span set to 0.2). Tristimulus scores brightness, hue and saturation (red chroma) were calculated from the resulting curve. Brightness was calculated as average reflectance, hue as wavelength at the reflectance midpoint and red chroma as a summed reflectance of 600–700 nm divided by a summed reflectance of 300–700 nm (Butler *et al.* 2011; Simons *et al.* 2012). Given that hue and red chroma were strongly intercorrelated ($r = 0.88$) and both shown moderate correlation with brightness (hue: $r = -0.62$; red chroma: $r = -0.67$), we only used red chroma in statistical analysis. Note that the conclusions would be unchanged, if brightness or hue was used instead (data not shown).

Statistical analysis

In order to discern the effects of within-individual age-related change and selective disappearance on age-related variation in tail length at the population level, we used linear mixed-effect models (LMM) implemented in R 3.4.3. In these models, tail length was set as a dependent variable and individual identity was included as a random factor. Only random effects on intercept were fitted as only one observation was available for those individuals that did not survived past the age of one year. We fitted the models separately for each sex to avoid

overly complicated model structure with higher-order interactions. We adopted two approaches to analyse data from two separate populations. First, we fitted population-specific models to test for age-related effects on tail length in each population separately. Second, we fitted joint models for both populations to test for both the average effects across populations and significant differences in effects between populations. In the joint models, populations were fitted as a continuous variable coded 0 (the CZ population) and 1 (the RO population) and centred, which allows to interpret the main effects of age and lifespan as average effect across populations. We introduced age as a cubic effect (third-order polynomial) in the models, because fitting age with only a quadratic age effect (second-order polynomial) may result in a false positive detection of senescence when phenotypic change is greater at an early age with subsequent levelling off (Bouwhuis *et al.* 2009). Non-linear effects were always fitted as orthogonal polynomials to avoid collinearity. We further introduced lifespan as one of the explanatory variables to test for the effect of selective disappearance with respect to tail length. Inclusion of both age and lifespan as the predictors in the model is important as it allows to discern within-individual changes in trait expression with age and the effect of selective disappearance (van de Pol & Verhulst 2006). In other words, this approach tests for the effect of within-individual change in analysed trait in the presence of selective disappearance and *vice versa*. The estimate of age-related changes in trait expression cannot be interpreted as sole effect of within-individual change when lifespan is not included among predictors as it also contains the between-individual effect of selective disappearance (van de Pol & Verhulst 2006). We also included the quadratic term for lifespan, as suggested by former studies and interpreted as resulting from a combination of selective disappearance and a trade-off in resource allocation between trait expression and survival (Reid *et al.* 2003; Bouwhuis *et al.* 2009).

Although the aforesaid LMM approach can test for selective disappearance, it cannot test for stabilizing or disruptive selection as the variable expressing survival (i.e. lifespan) is on the x-

axis and a phenotypic trait is on the y-axis. Hence, we also used a second approach to test for differential mortality with respect to tail length, the logistic discrete-time hazard models with logit link-function, death set as a binary variable (0 = survived to next year, 1 = died during the subsequent year) and age fitted as a categorical variable (Singer & Willett 2003). We used this approach rather than Cox proportional hazards modelling, because performance of the latter is low when there are only few discrete-time periods with high number of deaths in each (Singer & Willett 2003). As tail length was highly repeatable when age and population differences were controlled for (LMM-based repeatability [95% CI]: males: 0.90 [0.88–0.92]; females: 0.88 [0.85–0.90]), we used tail length in the first year of age and tested whether it predicts individual survival in subsequent years. First-year tail length was fitted together with its quadratic effect in these models to test for non-linear relationship between ornamentation and survival, which would be indicative of either stabilizing or disruptive selection. Again, we fitted separate models for each population and a joint model including observation from both populations. Population together with two-way interactions with age, tail length and year were included among predictor terms in the joint model to test for between-population differences. Age was included as centred dummy variables. Population was centred in the same way as in LMM models, hence the main effects are interpreted as the average effects across populations. We only used age classes one to four in CZ males, CZ females and RO females and age classes one to three in RO males as probability of mortality in older age classes cannot be plausibly estimated due to the low number of individuals surviving to the late age. In a joint model fitted with male data from both populations, we only used age classes one to three to prevent rank deficiency. The models controlled for variation in mortality between years by including calendar year as a continuous variable with quadratic effect. The quadratic effect of year was supported over the linear and cubic effects, as well as over including this variable as a factor,

according to an AICc-based model comparison using the joint model as a background model (Table S1 in Supplementary Information).

Our dataset included a subset of CZ males that underwent experimental manipulation of tail length and ventral colouration. We used this subset to test the costs of tail length manipulation in terms of both survival and tail streamer length grown during the subsequent moult. The effect of tail length manipulation was tested using logistic discrete-time hazard model with manipulation group (control, elongated, short) fitted as a predictor. We also included ventral colouration (control, darkened) manipulation to control for its effect. The model also controlled for age and year effects in the same way as above (i.e. included as categorical and quadratic terms, respectively). We also included both pre-manipulation tail length and ventral colouration to control for potential bias due to phenotypic differences between manipulation groups. Indeed, our approach of swapping tail streamers between males resulted in males from elongated and shortened group having pre-manipulation tail length shorter and longer, respectively, compared to control males (elongated: $\beta = -3.14 \pm 0.85$, $t = -3.69$, $P < 0.001$; shortened: $\beta = 3.19 \pm 0.74$, $t = -4.30$, $P < 0.001$). In contrast, there was no difference in pre-manipulation redness of the ventral colouration between darkened and control males ($\beta = 0.004 \pm 0.004$, $t = 0.95$, $P = 0.34$). We also tested for the previously reported differential effect of tail manipulation on short- and long-tailed males (Møller & de Lope 1994) by including interaction between tail manipulation and pre-manipulation tail length. The significance of the predictor terms was tested using likelihood ratio test (LRT).

Experimental manipulation of tail length was previously reported to affect length of the tail streamers grown during subsequent moult. We tested this using LMM with tail length in the year after experimental manipulation as a dependent variable and individual identity as a random effect. Manipulation of tail length and ventral colouration were fitted as categorical fixed effects and pre-manipulation tail length as a covariate. We also introduced a quadratic

term for age to control for age-related changes in tail length. The significance of the predictor terms was tested using LRT based on maximum likelihood estimation. Ornament manipulations showed no significant effect in any of the models in this study (see Results and Table S2 and S3 in Supplementary Information), hence we only present models without these terms, except the models intended to test the manipulation effects.

Results

Tail length associations with age and lifespan

At the population level, tail length monotonically increased with age in both male and females with highest increase between the first and the second year of life and a slowdown in subsequent years (Figure 1a, b). In order to discern relative contribution of within-individual change and selective disappearance to this pattern, we fitted LMM with tail length set as dependent variable and both age and lifespan together with their third- and second-order polynomials, respectively, as predictors.

This approach revealed significant within-individual age-dependent variation in tail length in both sexes with similar dynamics to the one at the population level; i.e. highest increase in length between the first and the second year of life (steeper in the CZ population) and a slowdown in subsequent years (Table 1). Plotting the individual inter-annual changes in tail length showed no evidence of senescence in this trait in any of the population or sex (Figure 1c, d). The lack of senescence in tail length was further supported by the positive coefficient of the third-order polynomial of age in all the models, though significant only in CZ males and in the joint model for males from both populations. To investigate how removing the third-order polynomial of age from the model affects interpretation of the results, we further fitted a model with only quadratic age effect and plotted the predicted values of both models. In contrast to both the model with cubic age effect and calculated individual inter-annual changes in tail

length, the results of a model including only quadratic age effect could be interpreted as indicating senescence in tail length in both sexes (Figure 1e, f). This suggests that including only second-order polynomial of age in a model could result in a false positive detection of senescence.

The LMM further tested for selective disappearance with respect to tail length. Across populations, tail length was significantly correlated with lifespan suggesting selective disappearance of short-tailed males, but not females (Table 1). Separate models for each population showed that although the estimate was also positive in RO males, the relationship between tail length and lifespan was only significant in the CZ population (Table 1; Figure 2). The non-significant interaction between lifespan and population suggests, however, that the association between tail length and lifespan does not differ between CZ and RO males.

First-year tail length and survival

We further tested whether tail length measured in the first year of age predicts individual survival in following years using logistic discrete-time hazard models. In CZ males, there was a significant second-order polynomial effect of first-year tail length suggesting that the positive relationship between tail length and survival indicated by LMM approach is reversed in males with extremely long tails (Table 2; Figure 3). This quadratic effect was only marginally non-significant when effect of ornament manipulations has been controlled for ($P = 0.055$; Table S3 in Supplementary Information), however, which suggests that the increase in mortality in long-tailed males may be mild (see also confidence intervals in Figure 3). In the RO population, this effect was not significant, though the joint model indicated no significant difference between the populations. In the CZ population, best survivors were males having first-year tail length 5 mm longer than the population median (114 mm vs. 109 mm). This provides an evidence for overall directional survival selection for longer tails (as evidenced also by LMM approach),

combined with weak stabilizing selection acting on males with extremely long tails. In contrast, no such selective mortality was found in females.

Effect of tail length manipulation on male mortality

To assess costs of tail streamers in terms of survival, we tested whether experimental manipulations of tail length affected the probability of male survival to the next year in the CZ population. Tail length manipulation was combined with experimental darkening of ventral colouration in 2014 and 2015, hence we also included this effect in the model. We found no support for male tail streamers or ventral colouration being costly in terms of survival, as experimental manipulation of neither tail length nor ventral colouration showed a significant effect on male survival probability (tail length manipulation: LRT: $\Delta G^2 = 1.33$, $\Delta df = 2$, $P = 0.51$; effect of tail elongation: $\beta = 0.34 \pm 0.33$, $P = 0.31$; effect of tail shortening: $\beta = -0.05 \pm 0.32$, $P = 0.86$; manipulation of ventral colouration: LRT: $\Delta G^2 = 2.55$, $\Delta df = 1$, $P = 0.11$; effect of darkening: $\beta = -0.68 \pm 0.43$, $P = 0.11$). An addition of an interaction between pre-manipulation tail length and tail length manipulation to the model did not support previously reported differential survival of naturally short- and long-tailed males after manipulation (LRT: $\Delta G^2 = 0.75$, $\Delta df = 2$, $P = 0.69$; effect of pre-manipulation tail length in elongated males: $\beta = -0.027 \pm 0.039$, $P = 0.48$; effect of pre-manipulation tail length in shortened males: $\beta = -0.027 \pm 0.039$, $P = 0.49$).

Effect of tail length manipulation on tail streamer growth during subsequent moult

We further tested whether experimental manipulation of tail streamer length influences the length of male tail streamers grown during subsequent moult using LMM models with tail length in the next year as a dependent variable. Age and pre-manipulation tail length were controlled for in the model. Again, there was no support for the tail streamers being costly as experimental manipulation of neither tail length nor ventral colouration showed a significant

effect on the length of male tail streamers in the next year (tail length manipulation: LRT: $\Delta G^2 = 4.01$, $\Delta df = 2$, $P = 0.13$; effect of tail elongation: $\beta = -0.88 \pm 0.85$, $P = 0.31$; effect of tail shortening: $\beta = -1.47 \pm 0.77$, $P = 0.072$; manipulation of ventral colouration: LRT: $\Delta G^2 = 0.29$, $\Delta df = 1$, $P = 0.59$; effect of darkening: $\beta = 0.43 \pm 0.84$, $P = 0.61$). An addition of an interaction between tail length manipulation and pre-manipulation tail length revealed no significant differential effect of ornament manipulation in birds with differing tail length (LRT: $\Delta G^2 = 0.19$, $\Delta df = 2$, $P = 0.90$; effect of pre-manipulation tail length in elongated males: $\beta = -0.067 \pm 0.121$, $t = 0.55$; effect of pre-manipulation tail length in shortened males: $\beta = -0.015 \pm 0.105$, $t = -0.14$).

Discussion

One of the main aims of our study was to test for a condition-dependence of tail streamer length in two European barn swallow populations, namely the differential mortality related to the expression of this secondary sexual trait. Using two different statistical approaches, we detected such selective mortality in males, but not females. The first approach, based on an inclusion of lifespan among predictors of tail length in the linear mixed-effect model (van de Pol & Verhulst 2006), showed a positive linear relationship between male tail length and lifespan indicating that males with longer tails live longer. Although this relationship was only significant in CZ males when each population was analysed in a separate model, the joint model supported its existence across populations. In the CZ population, such selective disappearance of short-tailed males was also supported by the second approach, the logistic discrete-time hazard modelling. This approach indicated, however, that the relationship between tail length and mortality is not linear and that males with extremely long tails also suffer higher mortality. In the CZ population, the highest survival probability was observed in males with tails 5 mm longer than the population median, providing an evidence for directional selection combined with stabilizing selection acting on males with extremely long tails. The

quadratic effect of tail length on survival has become marginally non-significant when effect of ornament manipulation was controlled for in the model. Together with positive correlation between tail length and lifespan evidenced by LMM approach, this suggests that the increase in mortality in long-tailed males may only be mild. The non-linear relationship between tail length and mortality was not supported in the RO population, perhaps due to either lower number of observations or between-population differences. The differences between populations were not supported by the joint model, however, as the interaction between population and tail length was non-significant. Nevertheless, both approaches used in our study indicated directional survival selection on tail length in males but not females, either across populations (LMM approach) or in the CZ population only (discrete-time hazard modelling), which could provide one of the evolutionary mechanisms explaining maintenance of elongated tail streamers in barn swallow males.

Previous studies in different barn swallow populations provided contrasting results reporting either negative (Møller & Szep 2002; Balbontín *et al.* 2011) or positive (Møller 1991, 1994a; Saino *et al.* 1997; Romano *et al.* 2017) correlation between tail length and survival, with our results supporting the latter. Such a discrepancy suggests that the relationship between tail length and survival might not be universal across barn swallow populations. This may relate to differing conditions experienced by various populations due to, for example, between-population differences in migration routes or wintering grounds (Ambrosini *et al.* 2009; Klvaňa *et al.* 2018). Alternatively, such contrasting results may be due to resource availability being variable between populations or among years since resource acquisition can alter relationship between fitness components that are traded off against each other (Van Noordwijk & de Jong 1986). Our data suggest that at least the extremely long tail streamers are indeed traded off against survival, though our manipulation experiment suggests the survival costs of tail streamers are low. We can also speculate that the interplay between resource acquisition and

allocation can possibly underpin the non-linear relationship between tail length and survival observed in our study. As resource acquisition is limited (either due to limited resource availability or due to physiological limits in resource acquisition) available resources may be sufficient in short-tailed males for both ornament expression and survival, resulting in their positive correlation, but may be limiting in males with extremely long tails. In addition, extremely long tails can even interfere with resource acquisition due to reduced manoeuvrability, an important quality for aerial foraging (Buchanan & Evans 2000).

Our results showing a link between male tail streamer length and survival support condition dependence of this sexual trait, at least in the CZ population. This is in line with a previous meta-analysis indicating that most sexually selected traits may serve as viability indicators (Jennions *et al.* 2001). The positive correlation between male tail length and viability would speak against the pure Fisherian runaway process being responsible for evolution of this sexual trait in the barn swallow (Lande 1981). Nevertheless, a unifying model has been proposed suggesting that pure Fisherian process and indicator mechanism are opposite ends of one sexual selection continuum (Kokko *et al.* 2002). According to this model, the direction of correlation between ornamentation and survival may depend on the costliness of the female choice, which determines the intensity of sexual selection that females impose on males. The model predicts that less costly female choice results in negative relationship between attractiveness and survival due to intense sexual selection, whereas more relaxed sexual selection resulting from high female choice costliness restores viability signalling function of a display (Kokko *et al.* 2002). Accordingly, positive correlation between tail length and viability observed in our study would imply high costs of female choice and, consequently, relaxed sexual selection on male tail length.

Our data confirm previously reported (Møller & De Lope 1999; Balbontín *et al.* 2011) non-linear within-individual increase in pre-senescent tail length with age with highest elongation

between the first and the second year of life and a slowdown in subsequent years. Therefore, the age-related increase in tail length observed at the population level is due to combination of within-individual increase in tail length and selective disappearance of short-tailed animals in our populations. While LMM fitted with a second-order polynomial function of age indicated senescence in tail length in late life, both the individual inter-annual differences and LMM fitted with a third-order polynomial of age showed no signs of senescence. This demonstrates that fitting models with only a quadratic age effect could result in a false positive detection of senescence in tested traits in cases where the change in trait expression is steeper in early life with subsequent slowdown (Bouwhuis *et al.* 2009). This could provide a possible explanation for a discrepancy between previous studies reporting senescence in tail length in the barn swallow (Møller & De Lope 1999; Balbontín *et al.* 2011) and our study finding no senescence in this trait, despite covering the same number of age classes (from one to six years of age in both sexes). Hence, our results suggest that second-order polynomial of age effect should be in general considered with caution when analysing senescence.

The observed within-individual increase in tail length with age is a pattern reported for a variety of sexual traits in many species including the barn swallow (Delhey & Kempenaers 2006; Nussey *et al.* 2009; Val *et al.* 2010; Balbontín *et al.* 2011; Evans *et al.* 2011; Kervinen *et al.* 2015). It has been suggested that older individuals with highly expressed sexual traits can signal their proven viability and are preferred in mate choice (Manning 1985). This is in accord with our observation from the CZ population, where older males are more successful in gaining extra-pair paternity (Michálková et al. unpublished manuscript). The within-individual increase in tail length in response to age is also in accord with a game theoretic model based on life-history theory predicting increasing investment in sexual ornamentation with decreasing residual reproductive value of an individual (Kokko 1997, 1998). Considering that ageing individuals should experience decline in physiological functions (López-Otín *et al.* 2013), even

maintaining a stable level of ornament expression toward the end of the life may be seen as an increasing investment in sexual signalling (Evans *et al.* 2011). In the barn swallow, such a physiological decline in old age has been documented in reproductive and immune functions (Møller & De Lope 1999; Saino *et al.* 2003). Hence, our data showing no senescence in tail length indicate that investment in sexual advertisement increases over the entire individual lifespan.

We further tested the costs of elongated male tail streamers using experimental manipulation of their length in the CZ population. In contrast to previous experiments done in different barn swallow populations (Møller & de Lope 1994; Saino *et al.* 1997), we found no significant effect of tail length manipulation on survival, despite using comparable sample size. There was also no indication of previously reported (Møller & de Lope 1994) differential survival of naturally long- and short-tailed males after manipulation as evidenced by non-significant interaction between tail manipulation and pre-manipulation tail length. We also found no effect of manipulations on the length of tail streamers grown during subsequent moult, which was also previously reported (Møller 1989). Such a discrepancy may have several possible explanations. First, the way we manipulated tail streamer length differed from previous studies. Instead of cutting out a proximal piece of tail streamer in shortened males and pasting it to the base of tail streamer in elongated males (Møller 1989; Møller & de Lope 1994), which results in both a displacement of white tail spots and unnatural proportions between wide and narrow parts of the streamer vane, we swapped whole feathers between males keeping the proportions natural (Bro-Jørgensen *et al.* 2007; Vortman *et al.* 2013; Safran *et al.* 2016). The extension of the wide part of streamer vane used in previous studies could result in feather being heavier and having different aerodynamic properties compared to streamers with natural proportions. Second, our approach needed only one joint per streamer where the feather parts were glued together instead of two, thus reducing the chance of a joint failure by 50%. This may be highly relevant as the

joint must hold from the time of manipulation to a subsequent moult at the wintering grounds and its failure would cause the distal streamer part falling off possibly increasing probability of mortality due to impaired manoeuvrability. Hence, by reducing the number of joints, we reduced the chance of such an artefactual mortality affecting our results. Third, our approach based on swapping tail streamers between males resulted in males from elongated group having shorter mean pre-manipulation tail length compared to males from shortened groups, whereas there was no such difference in previous studies (Møller 1989; Møller & de Lope 1994). Nevertheless, such a difference should actually strengthen the effect of manipulation in our study, at least in the elongated group, as short-tailed males are the ones expected to suffer the highest costs of tail elongation (Møller & de Lope 1994). Fourth, we only manipulated the tail length by 1.5 cm (2 SD in our population) instead of 2 cm used in previous studies (Møller 1989; Møller & de Lope 1994), which could be another possible cause of our failure to detect any significant effect on survival. Tail shortening or elongation by 1.5 cm is still a substantial alteration compared to the distribution of tail length variability, however, suggesting that costs of bearing tail streamers are at best mild. Inferring from our correlational data, the survival costs may only be significant in males with extremely long tail streamers.

The lack of significant costs of tail streamer expression could alternatively imply that honesty of condition-dependent signalling is not maintained primarily through tail streamer costs but through expression of this sexual trait being linked to physiological processes and signalling pathways underlying individual phenotypic quality (Hill 2011). For example, insulin/insulin-like signalling has been proposed as one of the pathways possibly linking growth of exaggerated structural sexual traits with body condition without assuming ornamentation costs (Emlen *et al.* 2012; Warren *et al.* 2013). Insulin/insulin-like signalling pathway interacts with growth hormone signalling pathway, androgens and glucocorticoids and it is involved in control of metabolism, somatic growth, reproduction and ageing (Dantzer & Swanson 2012).

Therefore, it represents an exciting and largely unexplored mechanism that could link exaggerated sexual traits to phenotypic quality without the need of trait costliness. Nevertheless, it should be noted that this mechanism does not exclude the possibility of exaggerated sexual traits being costly in their extreme values.

In summary, our data suggest that elongated tail streamers in barn swallow males indicates both survival and age, i.e. potential and proven viability, while only the association with age was observed in females The positive correlation between tail length and survival may attest either to relaxed sexual selection resulting from high costs of female choice (Kokko *et al.* 2002) or high resource availability in our populations (Van Noordwijk & de Jong 1986), enabling males to mitigate display costs. Alternatively, costliness may not be the main mechanism ensuring honesty in this sexual trait as our manipulation experiment suggest that costs of tail streamers are at best mild and only extremely long tails appear to be costly. Hence, the best fitting to our data may the hypothesis proposing that condition-dependence of sexual display is not mediated through its costs but through its expression being linked to metabolic and signalling pathways underlying individual phenotypic quality (Hill 2011). In addition, we found no support for senescence in tail length in either sex. We also demonstrated that including only second-order polynomial of age as a predictor in a model may result in false positive detection of senescence and should be interpreted with caution.

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References

Ambrosini, R., Møller, A.P. & Saino, N. (2009). A quantitative measure of migratory connectivity. *J. Theor. Biol.*, 257, 203–211.

Balbontín, J., de Lope, F., Hermosell, I.G., Mousseau, T.A. & Møller, A.P. (2011). Determinants of age-dependent change in a secondary sexual character. *J. Evol. Biol.*, 24, 440–448.

Bitton, P. & Dawson, R.D. (2008). Age-related differences in plumage characteristics of male tree swallows Tachycineta bicolor: hue and brightness signal different aspects of individual quality. *J. Avian Biol.*, 39, 446–452.

Bouwhuis, S., Sheldon, B.C., Verhulst, S. & Charmantier, A. (2009). Great tits growing old: selective disappearance and the partitioning of senescence to stages within the breeding cycle. *Proc. R. Soc. Lond. B Biol. Sci.*, 276, 2769–2777.

Bro-Jørgensen, J., Johnstone, R.A. & Evans, M.R. (2007). Uninformative exaggeration of male sexual ornaments in barn swallows. *Curr. Biol.*, 17, 850–855.

Brooks, R. (2000). Negative genetic correlation between male sexual attractiveness and survival. *Nature*, 406, 67–70.

Buchanan, K.L. & Evans, M.R. (2000). The effect of tail streamer length on aerodynamic performance in the barn swallow. *Behav. Ecol.*, 11, 228–238.

Butler, M.W., Toomey, M.B. & McGraw, K.J. (2011). How many color metrics do we need? Evaluating how different color-scoring procedures explain carotenoid pigment content in avian bare-part and plumage ornaments. *Behav. Ecol. Sociobiol.*, 65, 401–413.

Costanzo, A., Ambrosini, R., Caprioli, M., Gatti, E., Parolini, M., Canova, L., *et al.* (2017). Lifetime reproductive success, selection on lifespan, and multiple sexual ornaments in male European barn swallows. *Evol. Int. J. Org. Evol.*, 71, 2457–2468.

Dantzer, B. & Swanson, E.M. (2012). Mediation of vertebrate life histories via insulin-like growth factor-1. *Biol. Rev.*, 87, 414–429.

Delhey, K. & Kempenaers, B. (2006). Age differences in blue tit Parus caeruleus plumage colour: within-individual changes or colour-biased survival? *J. Avian Biol.*, 37, 339–348.

Emlen, D.J., Warren, I.A., Johns, A., Dworkin, I. & Lavine, L.C. (2012). A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. *Science*, 337, 860–864.

Evans, S.R., Gustafsson, L. & Sheldon, B.C. (2011). Divergent patterns of age-dependence in ornamental and reproductive traits in the collared flycatcher. *Evolution*, 65, 1623–1636.

Fülöp, A., Vágási, C.I. & Pap, P.L. (2017). Cohabitation with farm animals rather than breeding effort increases the infection with feather-associated bacteria in the barn swallow Hirundo rustica. *J. Avian Biol.*, 48, 1005–1014.

Getty, T. (1998). Handicap signalling: when fecundity and viability do not add up. *Anim. Behav.*, 56, 127–130.

Grafen, A. (1990). Biological signals as handicaps. *J. Theor. Biol.*, 144, 517–546.
Hill, G.E. (2011). Condition-dependent traits as signals of the functionality of vital cellular processes. *Ecol. Lett.*, 14, 625–634.
Jennions, M.D., Moller, A.P. & Petrie, M. (2001). Sexually selected traits and adult survival: A meta-analysis. *Q. Rev. Biol.*, 76, 3–36.
Kervinen, M., Lebigre, C., Alatalo, R.V., Siitari, H. & Soulsbury, C.D. (2015). Life-history differences in age-dependent expressions of multiple ornaments and behaviors in a lekking bird. *Am. Nat.*, 185, 13–27.
Klvaňa, P., Cepák, J., Munclinger, P., Michálková, R., Tomášek, O. & Albrecht, T. (2018). Around the Mediterranean: an extreme example of loop migration in a long-distance migratory passerine. *J. Avian Biol.*, 49, n/a-n/a.
Kokko, H. (1997). Evolutionarily stable strategies of age-dependent sexual advertisement. *Behav. Ecol. Sociobiol.*, 41, 99–107.
Kokko, H. (1998). Good genes, old age and life-history trade-offs. *Evol. Ecol.*, 12, 739–750.
Kokko, H., Brooks, R., McNamara, J. & Houston, A. (2002). The sexual selection continuum. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.*, 269, 1331–1340.
Lande, R. (1981). Models of speciation by sexual selection on polygenic traits. *Proc. Natl. Acad. Sci. U. S. A.*, 78, 3721–3725.
López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M. & Kroemer, G. (2013). The hallmarks of aging. *Cell*, 153, 1194–1217.
Maia, R., Eliason, C.M., Bitton, P.-P., Doucet, S.M. & Shawkey, M.D. (2013). pavo: an R package for the analysis, visualization and organization of spectral data. *Methods Ecol. Evol.*, 4, 906–913.
Manning, J.T. (1985). Choosy females and correlates of male age. *J. Theor. Biol.*, 116, 349–354.
Møller, A. & Szep, T. (2002). Survival rate of adult barn swallows Hirundo rustica in relation to sexual selection and reproduction. *Ecology*, 83, 2220–2228.
Møller, A.P. (1989). Viability costs of male tail ornaments in a swallow. *Nature*, 339, 132–135.
Møller, A.P. (1991). Viability is positively related to degree of ornamentation in male swallows. *Proc R Soc Lond B*, 243, 145–148.
Møller, A.P. (1994a). Male ornament size as a reliable cue to enhanced offspring viability in the barn swallow. *Proc. Natl. Acad. Sci.*, 91, 6929–6932.
Møller, A.P. (1994b). *Sexual Selection and the Barn Swallow*. Oxford University Press.
Møller, A.P. & De Lope, F. (1999). Senescence in a short-lived migratory bird: age-dependent morphology, migration, reproduction and parasitism. *J. Anim. Ecol.*, 68, 163–171.
Møller, A.P. & de Lope, F. (1994). Differential costs of a secondary sexual character: an experimental test of the handicap principle. *Evolution*, 48, 1676–1683.
Nussey, D., Coulson, T., Festa-Bianchet, M. & Gaillard, J. (2008). Measuring senescence in wild animal populations: towards a longitudinal approach. *Funct. Ecol.*, 22, 393–406.
Nussey, D.H., Kruuk, L.E.B., Morris, A., Clements, M.N., Pemberton, J.M. & Clutton-Brock, T.H. (2009). Inter- and intrasexual variation in aging patterns across reproductive traits in a wild red deer population. *Am. Nat.*, 174, 342–357.
Pap, P.L., Tökölyi, J. & Szép, T. (2005). Frequency and consequences of feather holes in Barn Swallows Hirundo rustica. *Ibis*, 147, 169–175.
Quesada, J. & Senar, J.C. (2006). Comparing plumage colour measurements obtained directly from live birds and from collected feathers: the case of the great tit, Parus major. *J. Avian Biol.*, 37, 609–616.
R Core Team. (2017). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. http://www.R-project.org/, Vienna, Austria.

Reid, J., Bignal, E., Bignal, S., McCracken, D. & Monaghan, P. (2003). Age-specific reproductive performance in red-billed choughs Pyrrhocorax pyrrhocorax: patterns and processes in a natural population. *J. Anim. Ecol.*, 72, 765–776.

Romano, A., Saino, N. & Møller, A.P. (2017). Viability and expression of sexual ornaments in the barn swallow Hirundo rustica: a meta-analysis. *J. Evol. Biol.*, 30, 1929–1935.

Safran, R.J., Vortman, Y., Jenkins, B.R., Hubbard, J.K., Wilkins, M.R., Bradley, R.J., *et al.* (2016). The maintenance of phenotypic divergence through sexual selection: An experimental study in barn swallows *Hirundo rustica*. *Evolution*, 70, 2074–2084.

Saino, N., Bolzern, A.M. & Møller, A.P. (1997). Immunocompetence, ornamentation, and viability of male barn swallows (Hirundo rustica). *Proc. Natl. Acad. Sci.*, 94, 549–552.

Saino, N., Calza, S., Ninni, P. & Møller, A.P. (1999). Barn swallows trade survival against offspring condition and immunocompetence. *J. Anim. Ecol.*, 68, 999–1009.

Saino, N., Ferrari, R., Romano, M., Rubolini, D. & Moller, A. (2003). Humoral immune response in relation to senescence, sex and sexual ornamentation in the barn swallow (Hirundo rustica). *J. Evol. Biol.*, 16, 1127–1134.

Schaub, M. & Von Hirschheydt, J. (2009). Effect of current reproduction on apparent survival, breeding dispersal, and future reproduction in barn swallows assessed by multistate capture–recapture models. *J. Anim. Ecol.*, 78, 625–635.

Simons, M.J.P., Briga, M., Koetsier, E., Folkertsma, R., Wubs, M.D., Dijkstra, C., *et al.* (2012). Bill redness is positively associated with reproduction and survival in male and female zebra finches. *PLOS ONE*, 7.

Simons, M.J.P., Briga, M. & Verhulst, S. (2016). Stabilizing survival selection on presenescent expression of a sexual ornament followed by a terminal decline. *J. Evol. Biol.*, 29, 1368–1378.

Singer, J.D. & Willett, J.B. (2003). *Applied Longitudinal Data Analysis: Modeling Change and Event Occurrence*. Oxford University Press, New York, US-NY.

Val, E. del, Quesada, J. & Senar, J.C. (2010). Age-related differences in a carotenoid-based coloration trait are due to within-individual changes in great tits Parus major. *Ardea*, 98, 179–184.

Van Noordwijk, A.J. & de Jong, G. (1986). Acquisition and allocation of resources: their influence on variation in life-history tactics. *Am. Nat.*, 128, 137–142.

van de Pol, M. & Verhulst, S. (2006). Age-dependent traits: a new statistical model to separate within- and between-individual effects. *Am. Nat.*, 167, 766–773.

Vortman, Y., Lotem, A., Dor, R., Lovette, I. & Safran, R.J. (2013). Multiple sexual signals and behavioral reproductive isolation in a diverging population. *Am. Nat.*, 182, 514–523.

Warren, I.A., Gotoh, H., Dworkin, I.M., Emlen, D.J. & Lavine, L.C. (2013). A general mechanism for conditional expression of exaggerated sexually-selected traits. *Bioessays*, 35, 889–899.

Zahavi, A. (1975). Mate selection: selection for a handicap. *J. Theor. Biol.*, 53, 205–214.

Table 1. Linear mixed-effect models estimating effects of age and selective disappearance on tail length. Population (0 = CZ, 1 = RO) was centred, hence the main age and lifespan effects represent average effects across populations.

Parameter	Estimate	SE	*t*	*P*	Estimate	SE	*t*	*P*
	Males, both populations *n = 479 individuals, 703 observations*				*Females, both populations* *n = 413 individuals, 628 observations*			
Age	**63.81**	**3.84**	**16.62**	**<0.001**	**41.77**	**2.77**	**15.08**	**<0.001**
Age^2	**−23.37**	**3.24**	**−7.20**	**<0.001**	**−13.32**	**2.48**	**−5.37**	**<0.001**
Age^3	**9.96**	**3.10**	**3.21**	**0.002**	3.78	2.44	1.55	0.12
Lifespan	**35.27**	**16.66**	**2.12**	**0.035**	−4.75	9.62	−0.49	0.62
Lifespan^2	14.57	15.32	0.95	0.34	4.20	8.98	0.47	0.64
Population	**−4.52**	**1.05**	**−4.31**	**<0.001**	**−2.47**	**0.65**	**−3.82**	**<0.001**
Population × Age	**−21.73**	**9.53**	**−2.28**	**0.024**	2.82	6.29	0.45	0.65
Population × Age^2	14.95	8.55	1.75	0.082	−6.31	6.47	−0.98	0.33
Population × Age^3	−1.91	8.46	−0.23	0.82	6.60	6.52	1.01	0.31
Population × Lifespan	−8.42	44.08	−0.19	0.85	5.40	22.96	0.24	0.81
Population × Lifespan^2	14.11	39.55	0.36	0.72	−17.03	21.71	−0.78	0.43
	Males, CZ *n = 361 individuals, 527 observations*				*Females, CZ* *n = 273 individuals, 420 observations*			
Age	**60.42**	**3.69**	**16.35**	**<0.001**	**33.82**	**2.74**	**12.34**	**<0.001**
Age^2	**−23.59**	**2.91**	**−8.11**	**<0.001**	**−9.91**	**2.23**	**−4.44**	**<0.001**
Age^3	**9.51**	**2.71**	**3.51**	**<0.001**	1.68	2.15	0.78	0.44
Lifespan	**35.08**	**16.05**	**2.19**	**0.030**	−4.37	9.34	−0.47	0.64
Lifespan^2	9.48	14.73	0.64	0.52	7.97	8.57	0.93	0.35
	Males, RO *n = 118 individuals, 176 observations*				*Females, RO* *n = 140 individuals, 208 observations*			
Age	**23.54**	**3.54**	**6.64**	**<0.001**	**24.88**	**2.50**	**9.95**	**<0.001**
Age^2	**−7.34**	**2.75**	**−2.67**	**0.010**	**−10.17**	**2.09**	**−4.85**	**<0.001**
Age^3	2.94	2.52	1.17	0.25	2.99	2.02	1.48	0.14
Lifespan	7.86	11.94	0.66	0.51	0.94	9.12	0.10	0.92
Lifespan^2	8.81	11.27	0.78	0.44	−3.36	8.53	−0.39	0.70

Table 2. Logistic discrete-time hazard models testing whether adult tail length in the first year predicts survival throughout life. Death was included as a binary dependent variable. Population (0 = CZ, 1 = RO) and year (as dummy variables) were centred, hence the main effects of tail length represent average effects across populations.

Parameter	Estimate	SE	*z*	*P*	Estimate	SE	*z*	*P*
	Males, both populations				*Females, both populations*			
	n = 499 individuals, 697 observations				*n = 441 individuals, 666 observations*			
Year	4.12	2.39	1.73	0.084	**7.63**	**2.57**	**2.97**	**0.003**
Year^2	**−5.88**	**2.39**	**−2.46**	**0.014**	**−5.26**	**2.52**	**−2.09**	**0.037**
Age 2	**−0.42**	**0.20**	**−2.11**	**0.035**	−0.33	0.20	−1.62	0.11
Age 3	−0.01	0.36	−0.02	0.98	**−0.96**	**0.30**	**−3.16**	**0.002**
Age 4					0.17	0.46	0.38	0.70
Tail length	−2.46	2.58	−0.95	0.34	2.14	2.31	0.93	0.35
Tail length^2	4.52	2.53	1.79	0.073	2.71	2.31	1.17	0.24
Population	−0.06	0.24	−0.27	0.79	**−0.41**	**0.20**	**−2.11**	**0.035**
Population × Year	0.51	6.95	0.07	0.94	−7.81	5.87	−1.33	0.18
Population × Year^2	11.93	7.01	1.70	0.089	2.17	5.73	0.38	0.70
Population × Age 2	**0.99**	**0.48**	**2.06**	**0.039**	−0.10	0.42	−0.24	0.81
Population × Age 3	**2.47**	**1.14**	**2.18**	**0.029**	0.60	0.63	0.95	0.34
Population × Age 4					1.41	0.99	1.42	0.15
Population × Tail length	4.51	7.41	0.61	0.54	−6.27	4.75	−1.32	0.19
Population × Tail length^2	−4.89	6.12	−0.80	0.42	−0.49	4.83	−0.10	0.92
	Males, CZ				*Females, CZ*			
	n = 384 individuals, 551 observations				*n = 282 individuals, 425 observations*			
Year	**4.41**	**2.14**	**2.06**	**0.039**	**9.51**	**2.25**	**4.23**	**<0.001**
Year^2	**−7.67**	**2.14**	**−3.58**	**<0.001**	**−5.02**	**2.26**	**−2.22**	**0.026**
Age 2	**−0.65**	**0.22**	**−2.89**	**0.004**	−0.29	0.26	−1.15	0.25
Age 3	**−0.58**	**0.34**	**−1.72**	**0.086**	**−1.17**	**0.38**	**−3.08**	**0.002**
Age 4	−0.69	0.53	−1.31	0.19	−0.34	0.53	−0.63	0.53
Tail length	−1.77	2.26	−0.78	0.43	3.94	2.26	1.74	0.081
Tail length^2	**4.81**	**2.35**	**2.04**	**0.041**	2.28	2.27	1.00	0.32
	Males, RO				*Females, RO*			
	n = 115 individuals, 162 observations				*n = 159 individuals, 241 observations*			
Year	2.73	2.17	1.26	0.21	0.22	2.18	0.10	0.92
Year^2	1.12	2.25	0.50	0.62	−1.62	2.12	−0.77	0.44
Age 2	0.34	0.42	0.80	0.42	−0.39	0.33	−1.17	0.24
Age 3	1.89	1.08	1.74	0.081	−0.58	0.50	−1.15	0.25
Age 4					1.07	0.84	1.28	0.20
Tail length	0.14	2.15	0.07	0.95	−1.60	2.07	−0.77	0.44
Tail length^2	0.30	2.11	0.14	0.89	1.30	2.11	0.62	0.54

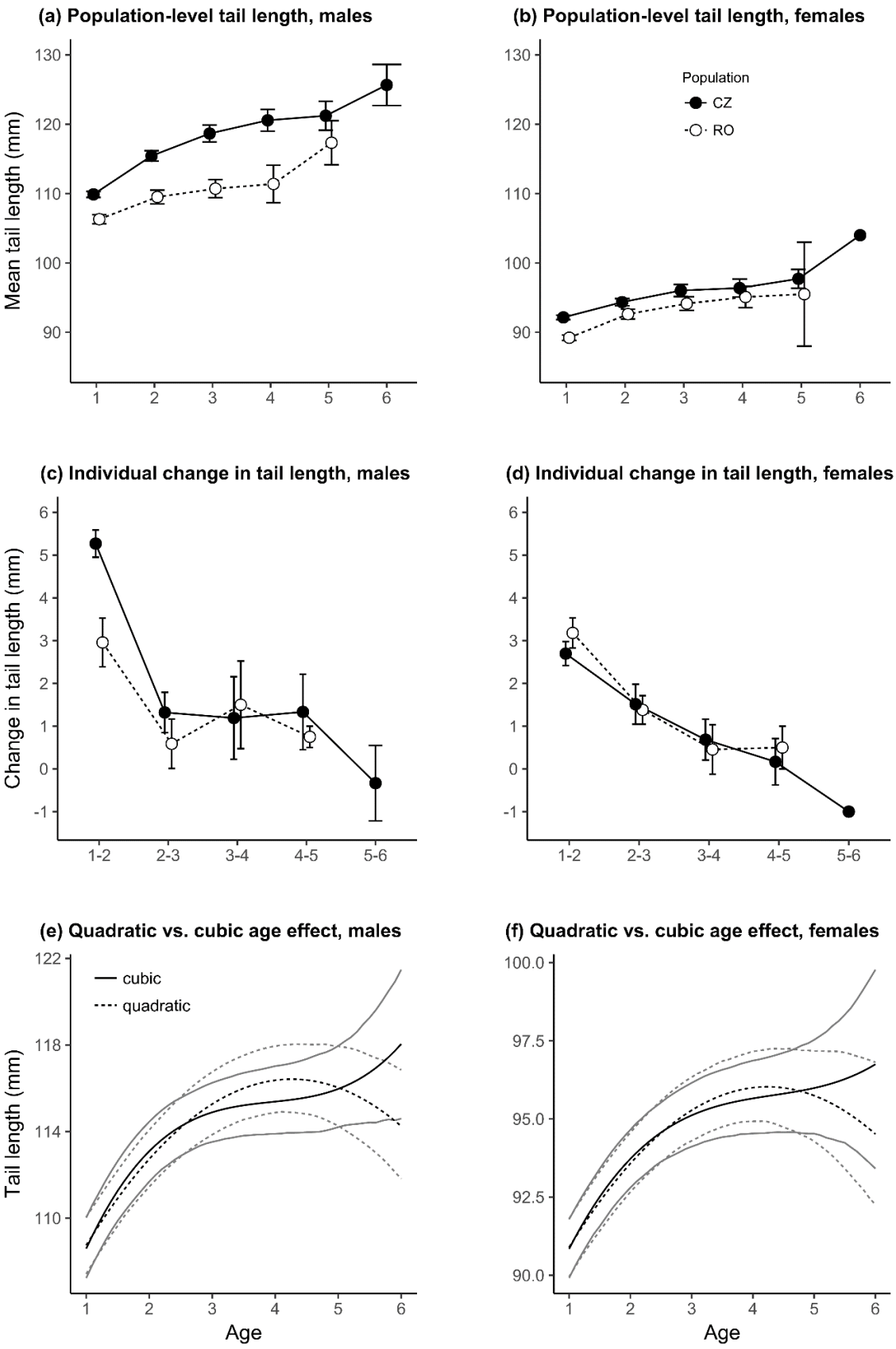

Figure 1. Age-related changes in tail length. Error bars denote standard errors of the mean (a-c) and grey lines 95% confidence intervals (e-f).

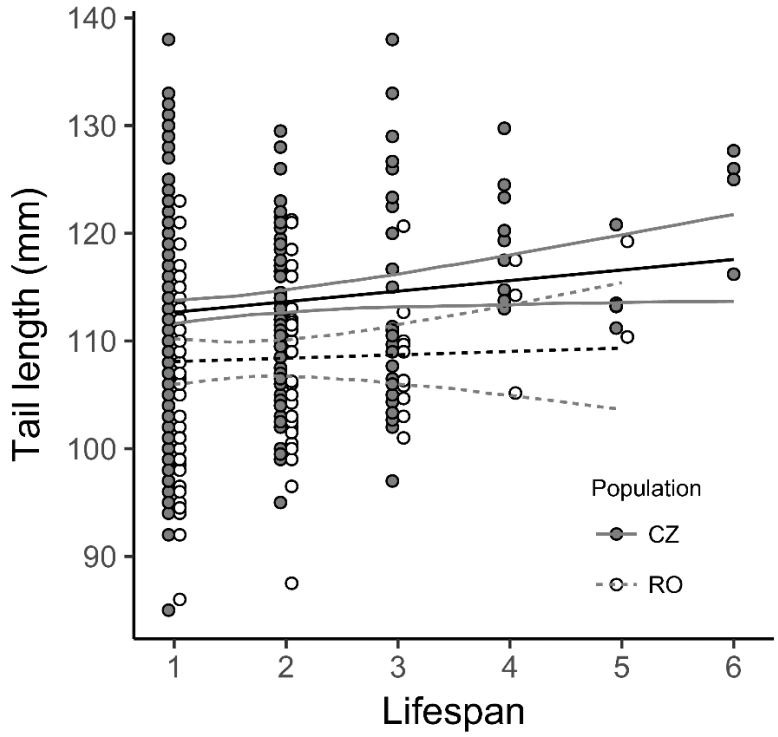

Figure 2. Relationship between tail length and lifespan. Lines are predicted values from the joint model with 95% confidence intervals.

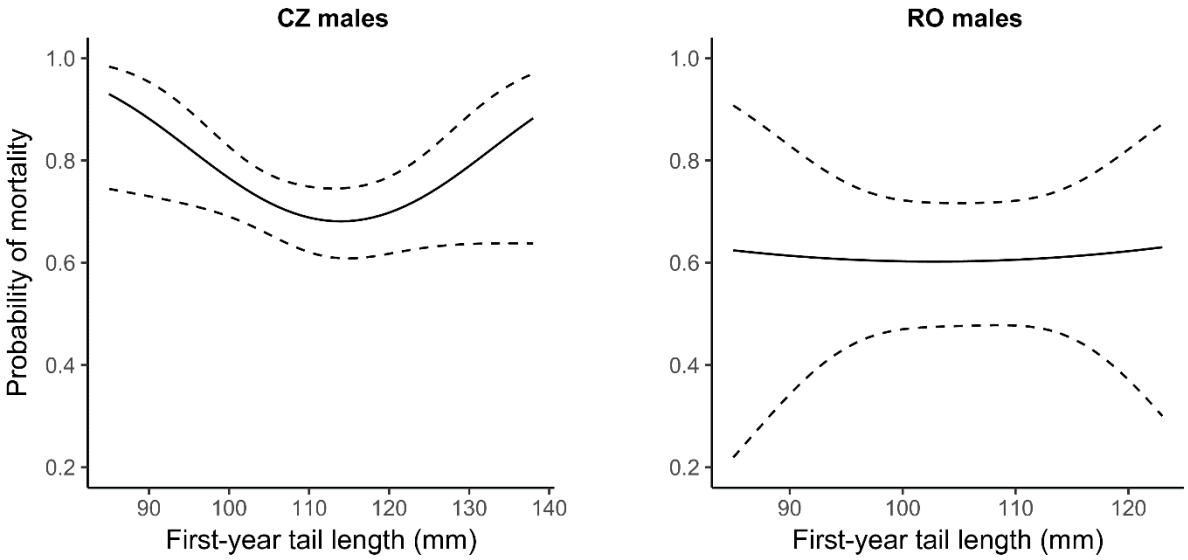

Figure 3. Probability of adult male mortality related to tail length in the first year of life. Shown are predicted values with 95% confidence intervals from the population-specific models from Table 2.

Supporting Information

for

Age-dependence and viability signalling function of tail streamer length in the European barn swallow

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Contents

Table S1. Candidate logistic discrete-time hazard models with different parametrisation calendar year. Compared are models including calendar year as either a factor or a continuous variable with linear, quadratic or cubic effect. All the models included population, age as a factor, quadratic term for tail length in the first year of life and two-way interactions of population with all the other main effects (see also Table 2 in the main text).

Sex	Year	Parameters	logLik	AICc	ΔAICc	Weight
Males						
	quadratic	14	−438.9	906.4	0.00	0.795
	cubic	16	−438.7	910.1	3.73	0.123
	factor	19	−436.0	911.2	4.75	0.074
	linear	12	−445.7	915.8	9.35	0.007
Females						
	quadratic	16	−429.0	890.9	0.00	0.493
	linear	14	−431.8	892.2	1.31	0.255
	cubic	18	−427.8	892.7	1.75	0.206
	factor	21	−426.1	895.6	4.72	0.046

Table S2. Linear mixed-effect model estimating effects of age, selective disappearance and ornament manipulations on tail length in Czech males

Parameter	Estimate	SE	*t*	*P*
Age	**62.82**	**4.94**	**12.72**	**<0.001**
Age^2	**−26.56**	**3.31**	**−8.02**	**<0.001**
Age^3	**10.24**	**2.81**	**3.65**	**<0.001**
Lifespan	**35.85**	**16.06**	**2.23**	**0.027**
Lifespan^2	10.28	14.73	0.70	0.49
Tail manipulation				
None	0.27	0.63	0.43	0.67
Elongation	−1.03	0.85	−1.21	0.23
Shortening	−0.56	0.70	−0.80	0.42
Ventral colour darkening	1.25	0.95	1.31	0.19

Table S3. Logistic discrete-time hazard models testing whether adult tail length in the first year predicts survival throughout life, while controlling for ornament manipulations. Death was included as a binary dependent variable.

Parameter	Estimate	SE	z	P
Year	3.84	2.42	1.59	0.11
Year^2	**−9.00**	**2.24**	**−4.02**	**<0.001**
Age 2	**−0.63**	**0.23**	**−2.78**	**0.005**
Age 3	−0.55	0.34	−1.60	0.11
Age 4	−0.61	0.54	−1.14	0.25
Tail length	−0.75	2.43	−0.31	0.76
Tail length^2	4.60	2.39	1.92	0.055
Tail manipulation				
None	0.45	0.32	1.38	0.17
Elongation	0.46	0.27	1.67	0.09
Shortening	0.04	0.31	0.13	0.90
Ventral colour darkening	−0.35	0.36	−0.98	0.33

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Selection on multiple sexual signals in two Central and Eastern European populations of the barn swallow

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Abstract

Variation in intensity and targets of sexual selection on multiple traits has been suggested to play a major role in promoting phenotypic differentiation between populations, although the divergence in selection may depend on year, local conditions or age. In this study, we quantified sexual selection for two putative sexual signals across two Central and East European barn swallow (*Hirundo rustica rustica*) populations from Czech Republic and Romania over multiple years. We then related these differences in selection to variation in sexual characters among barn swallow populations. Our results show that tail length and ventral coloration vary between populations, sexes, and age classes (first-time breeders vs. experienced birds). We found that selection on tail length was stronger in first-time breeders than in experienced birds and in males than in females in the Romanian population, while these differences between age groups and sexes were weak in Czech birds. We suggest that the populational difference in selection on tail length might be related to the differences in breeding conditions. Our results show that ventral coloration is darker (i.e., has lower brightness) in the Romanian than in the Czech population, and in experienced birds and males compared with first-time breeders and females, respectively. The sexual difference in ventral coloration may suggest sexual selection on this trait, which is supported by the significant directional selection of ventral coloration in first-time breeding males on laying date. However, after controlling for the confounding effect

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of wing length and tarsus length, the partial directional selection gradient on this trait turned nonsignificant, suggesting that the advantage of dark ventral coloration in early breeding birds is determined by the correlated traits of body size. These findings show that ventral coloration may be advantageous over the breeding season, but the underlying mechanism of this relationship is not clarified.

KEYWORDS
barn swallow, *Hirundo rustica rustica*, sexual selection, tail length, ventral coloration

1 | INTRODUCTION

Variation in sexually selected traits—traits used in mate selection—have been suggested to play a major role in promoting the phenotypic differentiation between populations, which may ultimately lead to morphological diversity, reproductive isolation during population divergence, and species formation (Boul, Chris Funk, Darst, Cannatella, & Ryan, 2007; Coyne & Orr, 2004; Saetre et al., 1997). Divergence in sexual signals due to differential targets of female preference may explain apparent morphological differences among recently diverged populations and subspecies, and population structure may eventually evolve if divergent signals lead to assortative mating (van Doorn, Edelaar, & Weissing, 2009; Vortman, Lotem, Dor, Lovette, & Safran, 2013). A first step in understanding whether secondary sexual traits differ between populations as a function of divergent mate preference entails careful, longitudinal studies of how phenotype variation is associated with reproductive performance.

Multiple male ornamental traits typically co-occur and are presented to choosy females simultaneously, with expression and signaling information depending on their production and maintenance costs (Bro-Jørgensen, 2010). The divergence between populations in sexual signals can be related to the difference between signals in their production costs and sensitivity to environmental conditions (Candolin, 2003; Maan & Seehausen, 2011). Therefore, variation in sexual traits between populations may depend on year, because climatic conditions affect the expression of sexual traits and geographically distinct populations experience differences in conditions between years (Chaine & Lyon, 2008; Reudink et al., 2015; Saino et al., 2004). Thus, the reported variation in phenotypes between populations may depend on the scale and intensity of the study.

One classical example of sexual selection and the role of secondary sexual traits in phenotypic differentiation in the animal kingdom is the broadly distributed barn swallow, *Hirundo rustica*, which has six distinctive subspecies (Møller, 1994; Scordato & Safran, 2014). All subspecies are fairly similar in their ecological niche as they are aerial feeders and, except for two middle-eastern distributed subspecies, are highly migratory (Turner, 2006). Phenotypic differentiation between these subspecies is associated with divergent sexual selection among barn swallow subspecies (Safran et al., 2016; Scordato & Safran, 2014; Vortman et al., 2013). Moreover, the direction of phenotypic divergence is consistent with differences in sexual selection pressures among subspecies (Vortman et al., 2013; Wilkins et al., 2016). For example, the Israeli *H. r. transitiva* subspecies, which is characterized with dark ventral plumage, experiences directional selection for darker plumage. Similarly, the European *H. r. rustica*, which has the longest tail feathers of any subspecies, experiences directional selection for elongated tail feathers (Møller et al., 2006; Wilkins et al., 2016). In completion of this and other observational studies (for review see Scordato and Safran (2014)), experimental results show that dark coloration in North American and Israeli populations and the longer streamer length in Israeli populations are associated with increased reproductive outcomes (Safran et al., 2016), indicating that sexual selection favors different combinations of the same traits in recently diverged, yet geographically isolated populations of barn swallows.

One of six well-characterized subspecies within the larger *H. rustica* complex, the European barn swallow, *H. r. rustica*, has been the subject of intense research over the last thirty years, with an emphasis on sexual selection (Romano, Costanzo, Rubolini, Saino, & Møller, 2017). Previous research across 22 European and North African populations of *H. r. rustica*, not sampled here, demonstrated consistent directional selection for elongated tail feathers, as the intensity of selection with respect to breeding date (a measure of fitness) was significantly correlated for tail length across populations (Møller et al., 2006). This work suggests that females mate preferentially with long-tailed males; however, the strength of selection on tail length differed significantly among populations. Importantly, geographical patterns of phenotypic selection predict current patterns of phenotypic variation among populations (Møller, 1994). However, a recent study on two Central and Eastern European populations of *H. r. rustica* from the Czech Republic (the same populations sampled here) and Turkey shows that the selection for long tail in male barn swallows is weak (Wilkins et al., 2016), suggesting a relatively reduced function of this trait in mate choice compared to other populations from Europe (e.g., Denmark, Italy, and Spain; (Romano et al., 2017). Albeit, the intensity of selection on sexual traits may be underestimated due to the confounding effect of age, since it appears that the selection on at least one trait, the tail length, differs between barn swallows breeding for the first time and experienced birds (Møller et al., 2006). The geographical variation in selection strength indicates that the function of the tail length in mate choice decreases in populations toward lower latitudes situated in the proximity of

H. r. transitiva subspecies found in Israel (Møller et al., 2006; Wilkins et al., 2016). Barn swallows belonging to *H. r. transitiva* are characterized by a different set of sexual characters (darker ventral coloration and shorter tail), and because of the absence of physical barriers between the two subspecies, admixture between the two may explain the displacement in sexual traits and reduced selection on tail length in Central and East European birds compared with westerly populations (Wilkins et al., 2016).

Besides the long tail, recent studies suggest that ventral coloration can be important in mate choice and reproductive isolation in the European population of *H. r. rustica*. A study on an Italian population suggests sexual dimorphism in coloration of ventral feathers (Parolini et al., 2017; Saino, Romano, Rubolini, Teplitsky, et al., 2013), though the intensity of coloration is smaller than in *H. r. transitiva* (Wilkins et al., 2016). On the same Italian population, darker males had significantly higher seasonal reproductive success (Parolini et al., 2017), and survival was higher in males with relatively higher eumelanin-to-pheomelanin ratio of ventral body feathers (Saino, Romano, Rubolini, Ambrosini, et al., 2013). However, ventral coloration did not predict the lifetime reproductive success of these birds (Costanzo, Ambrosini, Caprioli, Gatti, Parolini, Canova et al., 2017). It appears that sexual selection acts on the ventral coloration of females as well, because females with higher UV reflectance of their ventral plumage coloration were more promiscuous and the proportion of male offspring in a nest increased with maternal plumage darkness (Costanzo, Ambrosini, Caprioli, Gatti, Parolini, Romano et al., 2017; Romano et al., 2015). Contrary to these findings, the study on Czech and Turkish populations of *H. r. rustica* shows that the selection for dark ventral coloration in male barn swallows is weak (Wilkins et al., 2016), suggesting a reduced function of this trait in mate choice compared to the Italian population. Taken together, it is clear that the selection for large and colorful traits and their advantage in mate choice differs between populations. Therefore, studies that enable direct comparisons with other populations, which are possibly exposed to different sexual selection, will fill this gap in our knowledge about the potential role of sexual signals in morphological differentiation and reproductive isolation both within and among the subspecies of barn swallow.

In this study, we utilize two long-term datasets from two Central and Eastern European populations, which are closely located to the *H. r. transitiva* subspecies. Birds from these populations preferentially use the East-European migratory flyway (Klvaňa et al., 2018), which may possibly affect the magnitude of admixture with *H. r. transitiva* and separate from more westerly and southerly *H. r. rustica*. We use the data collected from these two populations to investigate the relative importance of sexual selection in phenotypic expression of two putative secondary sexual traits. Collectively, 1,204 measures were taken from 828 individuals over seven and eight years, respectively, from the Czech Republic (Central Europe) and Romania (Eastern Europe). We estimate selection differentials and partial selection differentials (controlling for body size) on laying date and secondary sexual traits of male and female barn swallows that have been shown to have a role in sexual selection in one or more of the geographical populations/subspecies of this species (Romano et al., 2017; Scordato & Safran, 2014). Specifically, using laying date as an estimate of fitness (Møller et al., 2006) we calculated selection on the length of the outermost tail feathers and on melanin-based coloration of the white to chestnut ventral plumage region of male and female barn swallows. We calculated selection on these phenotypic traits for both sexes to give an estimate for the difference between sexes, which can be a proxy of the strength of sexual selection. Further, because first-breeders and experienced birds differ in arrival date and consequently in their access to breeding sites (Møller, 1994), the strength of sexual selection may change with age. Therefore, we measured the strength of selection on multiple sexual traits separately for first-breeders and experienced barn swallows.

2 | MATERIAL AND METHODS

2.1 | Field methods

We studied barn swallows breeding at two populations located within the Třeboňsko Protected Landscape Area in South Bohemia (Doudlebia, Czech Republic) and in Cojocna village (central Transylvania, Romania) over eight (2010–2017) and seven years (2011–2017), respectively. Nests from the Czech population were located at two isolated farms (Šaloun, Lomnice nad Lužnicí and Hamr, Lužnice; Petrželková et al., 2015) where barn swallows breed in colonies of up to 40 pairs. Birds from Romania were located in stall buildings where they breed mostly solitarily or in small colonies of up to ten pairs (Fülöp, Vágási, & Pap, 2017). The number of nests we monitored in both populations varied between 50 and 120 each year. Barn swallows were systematically captured using mist nets or nest-traps over the course of the breeding season, and each individual was tagged with an aluminium ring (both populations) and marked with unique color combination of 1–2 colored plastic rings (Czech population), which allowed us to identify the position of their nest. Standard morphological measurements were taken from all individuals in all capture years, including length of both outermost tail feathers, wing and tarsus length. Tail length was expressed as the mean of the left and right, unbroken streamer. At least ten contour feathers were collected from the ventral region for later spectrometric color measurements (see below) from adult birds captured over the whole study period in the case of the Czech population, and in 2017 from the Romanian population. Sex was determined by visual examination of the presence of a brood patch (only females develop a brood patch) and for each nest, we examined nests every 4–5 days to determine the laying date (appearance of the first egg), clutch size, and brood size at days 1 and 10 to 12 posthatching. Barn swallow populations from both countries have been carefully monitored in a series of studies, with more than 95% of all breeding birds captured annually. Previous observations of barn swallows (Schaub & von Hirschheydt, 2009), and our own capture—recapture data, indicate high breeding fidelity for adults (Pap, Tökölyi, & Szép, 2005; Pap et al., 2018; Petrželková et al., 2015). Because of the high breeding site fidelity and constant ringing effort, individuals that were captured in any year between 2011 and 2017 for the Czech and 2012 and 2017

for the Romanian populations, and had not been captured as adults in the previous year, could be assumed to be 1-year-old individuals at their first breeding season. Immigrating from colonies outsides our study area was rare, except in rare cases when they were local recruits (i.e., individuals that were ringed as nestlings at the studied population, allowing us to directly determine their age). We could thus categorize age in all breeding birds as 1-year-old (hereafter first-time breeders) or more than 1-year-old (hereafter experienced birds) except for the first study year, when aging was not possible. Therefore, the data from the first study year (2010 for Czech and 2011 for the Romanian population) were excluded from all subsequent analyses.

2.2 | Feather measurements

Samples of plumage were removed by gently plucking at least ten feathers from the ventral body region. Ten feathers were arranged together and fixed on a white paper index cards to achieve a layer equivalent to real ordering of feathers on the body of the bird. The reflectance of these feathers was measured with a spectrometer, model AvaSpec 2048 (Avantes, Netherlands), and an AvaLight-XE (Avantes, Netherlands) was used as a light source. The sensing head of the spectrometer was modified by using of self-made adapter that secure standardized light conditions by screening out the ambient light and maintains a constant distance of 3.5 mm between sensing head and measured sample. Each sample was measured three times at the distal part of the feather under an angle of 90°. The spectrometer was regularly calibrated after measurement of samples of each two individuals using WS-2 (Avantes, Netherlands) white color standard and absolute dark. Measurements were converted using the software program AvaSoft 7.8. Spectral data were analyzed in R 3.4.3 (R Development Core Team, 2017) using the R package pavo version 1.3.1 (Maia, Eliason, Bitton, Doucet, & Shawkey, 2013). Spectral data were trimmed to the range of wavelengths between 300 and 700 nanometers. Reflectance equivalent to wavelengths in integral numbers was established. Three measurements of each sample were averaged, and this average was smoothed by a span of 0.2. We calculated hue, chroma, and brightness for objective quantification of color variation. We used only brightness as the main color metric, because all three color metrics were previously found to be highly intercorrelated across the breast and belly of individual barn swallows (McGraw, Safran, & Wakamatsu, 2005; Safran & McGraw, 2004), and brightness is the most variable among-individual dimension of color in this body region. Lower brightness scores (% reflectance) indicate plumage color that appears darker, redder, and more saturated when compared to feathers with higher brightness scores, as shows a study on North American barn swallows (Safran & McGraw, 2004).

2.3 | Statistical analyses

We tested whether phenotypic traits and laying date differed among populations, sexes and age categories by building separate linear models (LMs) and linear mixed-effects models (LMEs; Bates, Mächler, Bolker, & Walker, 2015) for each of these parameters, including population, sex, age, year as fixed factors, and the identity of birds as a random factor. We report minimal models, in which all main effects and significant interactions were retained; these were all obtained using stepwise backward elimination based on the largest nonsignificant (>.05) p value.

We then calculated standardized directional (s) and quadratic (g) selection differentials and partial selection differentials (i.e., directional β and quadratic γ gradients, controlling for body size [see below]) in separate LMs and LMEs for each secondary sexual trait (tail length, ventral coloration), for each population, age group, and sex, following Lande and Arnold (1983) and (Brodie, Moore, & Janzen, 1995). Selection coefficients (differentials and gradients) provide an estimate of the change in phenotype in standard deviation units for a unit change in fitness. All phenotypic traits were scaled and centered by subtracting from each population mean and dividing by the standard deviation. Relative fitness was calculated by dividing individual values by the mean fitness for each population and used as the response variable.

Selection differentials were estimated as the regression coefficient of relative fitness on standardized phenotype, as described in Lande and Arnold (1983). Partial selection differentials (gradients), controlling for indirect selection on a trait due to selection on other traits (here wing and tarsus length) that are correlated with the secondary sexual trait in question, were estimated using multiple linear regression with the standardized fitness component (clutch initiation date) as the dependent variable and the standardized phenotypic characters as independent variables (Lande & Arnold, 1983). We decided to include both wing and tarsus length in these models, as they may reflect distinct allometric impacts of body size on the expression of secondary sexual traits (all correlations between tarsus and wing length, populations and sexes analyzed separately, Pearson's correlation, $p > .110$). We calculated differentials and gradients for the whole dataset including all years and in these models, the identity of individuals was included as a random factor, except for the brightness of the Romanian birds for which we had data only for one year and first-time breeders.

For tail streamers, the trait for which we had measurements across multiple breeding seasons, we also calculated selection coefficients separately for each year and these values were used in a GLM to analyse differences in selection between populations, sexes, and age. Quadratic selection estimates were calculated as the coefficients of the second-order polynomial term in models where secondary sexual signaling traits were included as explanatory variables separately. In order to insure the computation of unbiased selection coefficients based on polynomial effect sizes, quadratic estimates, and standard errors were doubled, following Stinchcombe, Agrawal, Hohenlohe, Arnold, and Blows (2008).

Our measure of fitness was clutch initiation date, as this fitness component correlates strongly with fledging success (Møller, 1994) and the effect size (the relationship between laying date and the expression of secondary sexual traits), a measure of the intensity of sexual selection, is large (Romano et al., 2017). Moreover, in a

previous study, sexual selection differentials calculated from female clutch initiation and fledging success were strongly correlated across 22 European and North African populations (Møller et al., 2006), reinforcing the suitability of this reproductive metric as a surrogate of fitness. For interpreting significance of selection coefficients, we corrected *P*-values for false discovery rate, as this is a superior method for controlling analysis-wide type I error when performing multiple comparisons (Benjamini, Drai, Elmer, Kafkafi, & Golani, 2001; Nakagawa, 2004). All statistical analyses were conducted in the R statistical environment, version R 3.5.3 (R Development Core Team, 2017).

3 | RESULTS

We tested the covariation between two putative secondary sexual traits separately for the Czech and Romanian populations and for males and females in linear and mixed-effect models, where age (fixed effect), identity of individuals and year (random effects) were controlled for. Tail length was not predicted by brightness in any of the populations and sexes ($\chi^2 < 3.54$, $N > 289$, $p > .060$), which indicates that these traits may show different aspects of signaling in this species.

Table 1 and Figure 1 demonstrate variation in two putative secondary sexual traits and in the laying date. Tail length was significantly higher in the Czech than in the Romanian population, and in experienced birds and males compared with first-time breeders and females, respectively. The significant age and sex interaction show that the increase in tail length with age was higher in males than in females. Brightness was lower in the Romanian than in the Czech population (darker color in the former), and in experienced birds and males compared with first-time breeders and females, respectively. Differences between age groups and sexes were similar between populations, as indicated by the absence of significant interaction between main factors. Barn swallows started to lay earlier in the Romanian than in the Czech population, and experienced birds bred earlier than yearlings in both females than males.

TABLE 1 Variation in phenotypic secondary sexual traits and laying date between populations, sexes, age classes (first-time breeders and experienced birds), and study years of barn swallows from Czech and Romanian populations. Results are from minimal fixed effect models. Significant effects are marked in bold

	df	χ^2	*p*
Tail length (*N* = 828 individuals)			
Population	1	30.12	**.0001**
Age	1	126.51	**<.0001**
Sex	1	1,085.33	**<.0001**
Population × Age	1	5.13	**.024**
Age × Sex	1	9.39	**.002**
Ventral feather brightness (*N* = 505 individuals)			
Population	1	9.62	**.002**
Age	1	4.04	**.044**
Sex	1	16.67	**<.0001**
Year	6	157.39	**<.0001**
Laying date (*N* = 828 individuals)			
Population	1	9.13	**.003**
Age	1	5.93	**.015**
Sex	1	4.88	**.027**
Year	6	9.97	.126
Age × Year	6	50.08	**<.0001**

Standardized directional selection differentials were significant or marginally significant for the tail length of the first-time breeding males from the Romanian and Czech populations, respectively (Table 2a). Similarly, brightness of the ventral feathers of the first-time breeding males from both populations was under significant directional selection, showing that barn swallows with long tails and dark ventral coloration started to breed earlier. Selection differentials on secondary sexual traits of experienced males from both populations were nonsignificant. Controlling for the correlative effect of wing length and tarsus length on secondary sexual traits proved that only the tail length of first-time breeding males from the Romanian population is under significant directional selection (Table 2b), after correction of the significance values for false discovery rate.

Standardized directional selection differentials were significant for the tail length of experienced female barn swallows from the Czech population and for the brightness of first-time breeding females from the Romanian population, respectively (birds with long tails and light ventral coloration bred earlier; Table 2a). After controlling for the correlative effect of wing length and tarsus length on secondary sexual traits, only the selection gradient on the tail length of experienced females from the Czech population remained significant (Table 2b).

The GLM of the difference between sexes, age categories, and populations in the standardized selection differential for the tail length shows that in the Czech population, selection on this trait is similar between age groups and between males and females (Table 3a, Figure 2). In the Romanian population, however, the selection differential on tail length was higher for the first-time breeders than experienced birds and for males than for females, respectively. When the selection differentials of the two populations were analyzed together, the significant population × age and population × sex interactions revealed differences between age groups and sexes among populations. That is, the difference between first-time breeders and experienced birds, and between males and females was significant for the Romanian population, while no difference was found between groups for the Czech population. Results on the partial selection differential on the tail length largely confirmed our former findings, although the difference between age categories for the Romanian population was less pronounced (Table 3b).

A GLM exploring the difference between sexes and age groups in the standardized directional selection differential for the brightness of

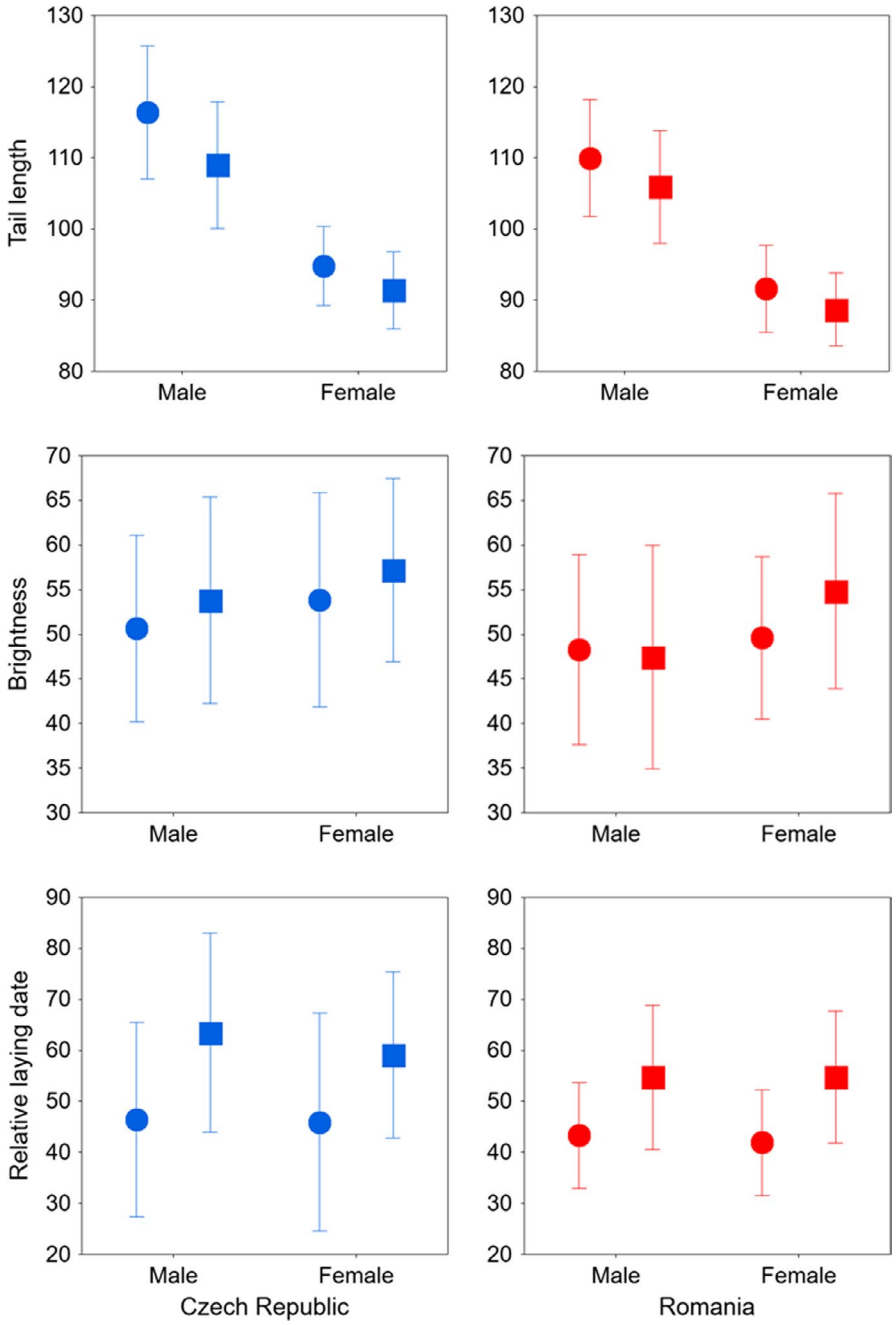

FIGURE 1 Representation of the variation in the two putative sexual traits and the laying date measured in experienced (circle) and first-time breeders (square) male and female barn swallows from the Czech (blue) and Romanian (red) populations. Mean ± *SD*

the Czech barn swallows, for which we had data from multiple years, shows that the selection on this trait is similar between age categories and between males and females (sex: $F = 0.00$, $p = .9910$; age: $F = 1.17$, $p = .2911$; sex × age interaction: $F = 0.54$, $p = .4707$; $df = 1,24$). Similar results were found when we used selection gradients accounting for size (sex: $F = 0.48$, $p = .4947$; age: $F = 1.65$, $p = .2118$; sex × age interaction: $F = 0.21$, $p = .6486$; $df = 1,24$).

Regression analyses of LMEs on second-order polynomial terms of putative secondary sexual traits showed no significant effect of the quadratic term after correction of the significance values for false discovery rate. Thus, there was no evidence for partial nonlinear selection differential on the traits that we measured (Table S1).

4 | DISCUSSION

Divergent selection on multiple signals can be associated with decoupled developmental pathways or the maintenance of genetic constrains (Bro-Jørgensen, 2010). Consistent with this prediction, we found that two putative secondary sexual traits, tail length and ventral coloration, in Czech and Romanian barn swallow populations were not significantly correlated, the magnitude of trait expression differed between the two populations, as did patterns of selection. In support of the prediction for the role of tail length in sexual selection in *H. r. rustica*, we found significant directional linear selection on this trait for both populations, in concordance with previous findings on other European populations (Costanzo,

TABLE 2 Standardized directional selection differentials and gradients (controlling for the effect of wing length and tarsus length) for the two putative sexual traits measured in first-time breeders and experienced male and female barn swallows from Czech (CZ) and Romanian (RO) populations. Significant effects (in case of partial directional selection differential after correcting for false discovery rate) are marked in bold

(A) Standardized directional selection differentials

	CZ				RO			
	N	*s (SE)*	*t*	*p*	*N*	*s (SE)*	*t*	*p*
Male								
Tail length								
First-time breeders	134	−0.043 (0.022)	−1.96	.0520	**138**	**−0.065 (0.021)**	**−3.11**	**.0023**
Experienced birds	156	−0.015 (0.030)	−0.49	.6268	159	−0.004 (0.017)	−0.21	.8349
Ventral feather brightness								
First-time breeders	**134**	**0.051 (0.022)**	**2.36**	**.0200**	**17**	**0.099 (0.042)**	**2.38**	**.0310**
Experienced birds	156	−0.051 (0.029)	−1.75	.0819	17	0.050 (0.048)	−1.03	.3204
Female								
Tail length								
First-time breeders	161	−0.023 (0.018)	−1.28	.2039	178	−0.019 (0.018)	−1.06	.2898
Experienced birds	**150**	**−0.098 (0.034)**	**−2.93**	**.0041**	128	0.024 (0.022)	1.10	.2718
Ventral feather brightness								
First-time breeders	161	0.015 (0.018)	0.86	.3934	**14**	**−0.076 (0.033)**	**−2.29**	**.0410**
Experienced birds	150	−0.015 (0.034)	−0.45	.6520	16	−0.048 (0.034)	−1.39	.1857

(B) Partial directional selection differentials, controlling for wing and tarsus length

	CZ					RO				
	N	*β (SE)*	*t*	*p*	*p adj*	*N*	*β (SE)*	*t*	*p*	*p adj*
Male										
Tail length										
First-time breeders	134	−0.038 (0.023)	−1.67	.0978	.1572	**138**	**−0.078 (0.023)**	**−3.43**	**.0008**	**.0024**
Experienced birds	156	−0.020 (0.033)	−0.62	.5397	.8096	159	0.001 (0.019)	0.04	.9666	.9666
Ventral feather brightness										
First-time breeders	134	0.050 (0.021)	2.33	.0213	.0639	17	0.099 (0.046)	2.17	.0493	.1480
Experienced birds	156	−0.050 (0.030)	−1.69	.0926	.2779	17	−0.050 (0.049)	−1.02	.3245	.4868
Female										
Tail length										
First-time breeders	161	−0.028 (0.019)	−1.47	.1444	.4053	178	−0.018 (0.019)	−0.94	.3497	.8480
Experienced birds	**150**	**−0.114 (0.038)**	**−2.98**	**.0035**	**.0104**	128	0.017 (0.023)	0.75	.4543	.6020
Ventral feather brightness										
First-time breeders	161	0.015 (0.018)	0.83	.4104	.6153	14	−0.071 (0.038)	−1.85	.0949	.2846
Experienced birds	150	−0.022 (0.035)	−0.63	.5316	.7975	16	−0.049 (0.046)	−1.07	.3054	.8996

Ambrosini, Caprioli, Gatti, Parolini, Canova et al., 2017; Møller et al., 2006). However, the magnitude of selection on tail length differed between populations, age groups, and sexes: Selection was stronger in first-time breeders than in experienced birds and in males than in females in the Romanian population, while differences between age groups and sexes were slight in Czech birds (Tables 2 and 3). Selection on tail length appeared to be independent of body size, because after controlling for the wing length and tarsus length, the difference between populations and groups remained the same. These results confirm previous findings, which have shown strong directional sexual selection on tail length in *H. r. rustica* males, but not females (Møller et al., 2006; Romano et al., 2017). Our findings suggest that the influence of selection may change with age and differ between closely related populations, despite a very low-level of genomic divergence between the Czech and Romanian barn swallows (Safran et al., 2016; Wilkins et al., 2016). One possible explanation for this populational difference in selection on tail length might be

TABLE 3 General linear model testing for differences in selection for tail length across populations, sexes and age categories (first-time breeders and experienced birds). Selection differentials (A) and gradients (B) were measured over subsequent years in Czech, CZ (2011–2017) and Romanian, RO (2012–2107) barn swallow populations. Significant effects are marked in bold

	CZ			RO			CZ + RO		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
(A) Factors influencing directional selection on tail streamers									
Age	1	1.48	.2355	**1**	**7.64**	**.0120**	1	0.12	.7330
Sex	1	1.08	.3091	**1**	**8.25**	**.0094**	1	0.29	.5910
Population	–			–			1	2.29	.1369
Age × Sex	1	2.86	.1036	1	0.22	.6417	1	2.92	.0941
Population × Age	–			–			**1**	**5.47**	**.0239**
Population × Sex	–			–			**1**	**5.04**	**.0298**
Error	24			20			45		
(B) Factors influencing directional selection on tail streamers, controlling for wing and tarsus length									
Age	1	**4.48**	**.0448**	**1**	1.71	.2063	1	0.93	.3403
Sex	1	1.09	.3079	**1**	**5.85**	**.0253**	1	0.27	.6056
Population	–			–			1	1.90	.1748
Age × Sex	1	0.27	.6090	1	0.63	.4373	1	2.92	.0941
Population × Age	–			–			**1**	**5.89**	**.0193**
Population × Sex	–			–			**1**	**4.79**	**.0339**
Error	24			20			45		

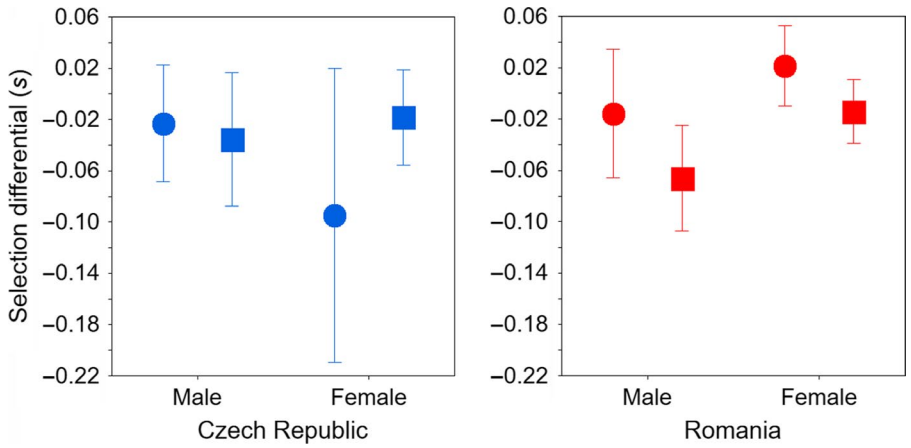

FIGURE 2 Difference between sexes and age categories (circle—experienced birds, square—first-time breeders) in the selection differential for the tail length measured over subsequent years in the Czech (2011–2017) and the Romanian (2012–2107) barn swallow population (for the statistics see Table 3). Mean ± *SD*

related to the differences in breeding conditions. Barn swallows in the Czech population breed in small colonies of up to 40 pairs, where the competition for nest sites can be low due to the large number of empty nests built over years and because the microhabitat of breeding sites from the same barns are similar, which render the nests similarly attractive. In the Romanian population, however, barn swallows usually breed solitarily in separate stall buildings, and birds preferentially occupy nests within barns populated with farm animals (Fülöp et al., 2017), possibly because of more favorable microhabitat conditions. The difference in colonial behavior between the two populations may explain the more intense selection on tail length for early laying in first-time breeding males from the Romanian population, where the number of favorable breeding sites is probably more limited, because experienced birds arrive first and occupy most of the preferred nest sites (PLP, pers. obs.). These differences can also be explained with the amount of variance in streamer length across age categories, because older birds are expected to be near their maximal character length and therefore there is not much variation on which selection to work. However, age groups were similar in homogeneity of variances (Levene's test: $F < 0.02$, $df < 1,288$, $p > .9251$; see Figure 1), which does not support this hypothesis.

We found that the selection on tail length is weak in experienced males and females in the Romanian, and in experienced males in the Czech population. The selection differentials for laying date on tail length fits within the range of selection previously observed on 22 European and North African barn swallow populations (Møller et al., 2006), albeit, the selection differential for experienced birds in the Romanian population is among the smallest of the studied populations of *H. r. rustica*. One explanation for the weak directional selection on tail length is related with the admixture of our populations to the proximal *H. r. transitiva*, which is characterized by a different set of sexual characters, and particularly by short tail length compared with *H. r. rustica* (Scordato & Safran, 2014; Wilkins et al., 2016). The present findings are in accordance with the evidence that substantial ongoing or recent historical gene flow is still present between *H. r. rustica* and *H. r. transitiva* (Dor et al., 2012), despite their apparent differences in morphology and life-history traits. Disentangling factors affecting the selection on tail length of barn swallows in different populations clearly deserves further investigations.

Ventral coloration was important in mate choice for different subspecies, specifically it was shown to be under directional selection at least in the North American *H. r. erythrogaster* and in *H. r. transitiva* from the Middle East (Safran et al., 2016; Vortman et al., 2013; Wilkins et al., 2016). Our results show that ventral coloration is more intense (i.e., has lower brightness) in the Romanian than in the Czech population, and in experienced birds and males compared with first-time breeders and females, respectively. The sexual difference in ventral coloration may suggest sexual selection on this trait, which is apparently supported by the significant directional selection of ventral coloration in first-time breeding males on laying date. After controlling for the confounding effect of wing length and tarsus length, the selection gradient on this trait lost significance, suggesting that the advantage of dark ventral coloration in early breeding birds is determined by the correlated traits of body size. These results are consistent with what has been found in an Italian population of barn swallows (Parolini et al., 2017; Saino, Romano, Rubolini, Teplitsky, et al., 2013). In this particular Italian population, darker males had significantly higher seasonal reproductive success, but coloration did not predict the lifetime reproductive success of the birds (Costanzo, Ambrosini, Caprioli, Gatti, Parolini, Canova et al., 2017; Parolini et al., 2017). These findings show that ventral coloration may give some advantages to the bearer over the breeding season, but the underlying mechanism of this relationship is not clarified (see (Costanzo, Ambrosini, Caprioli, Gatti, Parolini, Canova et al., 2017). Future studies on different barn swallow populations of *H. r. rustica*, distributed over a gradient from the nearest *H. r. transitiva* and experiencing different environmental conditions, may clarify the role and intensity of sexual selection in generating populational phenotypic differences of multiple secondary sexual traits.

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CONFLICT OF INTEREST

None declared.

AUTHOR'S CONTRIBUTIONS

PLP, AF, and RJS conceived the project; PLP, AF, MA, JC, RM, ANS, OT, CIV, OV, and TA collected the data; PLP and AF analyzed the data with input from RJS, MRW, OT, and TA; PLP wrote the paper with significant input from AF, MA, JC, RM, RJS, ANS, OT, CIV, OV, MRW, and TA. All authors gave final approval for publication.

DATA AVAILABILITY STATEMENT

Selection on multiple sexual signals in two Central and Eastern European populations of the. barn swallow: Dryad https://doi.org/10.5061/dryad.64p7k2f.

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REFERENCES

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48.

Benjamini, Y., Drai, D., Elmer, G., Kafkafi, N., & Golani, I. (2001). Controlling the false discovery rate in behavior genetics research. *Behavioural Brain Research*, *125*, 279–284. https://doi.org/10.1016/S0166-4328(01)00297-2

Boul, K. E., Chris Funk, W., Darst, C. R., Cannatella, D. C., & Ryan, M. J. (2007). Sexual selection drives speciation in an Amazonian frog. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 399–406. https://doi.org/10.1098/rspb.2006.3736

Brodie, E. D., Moore, A. J., & Janzen, F. J. (1995). Visualizing and quantifying natural selection. *Trends in Ecology & Evolution*, *10*, 313–318. https://doi.org/10.1016/S0169-5347(00)89117-X

Bro-Jørgensen, J. (2010). Dynamics of multiple signalling systems: Animal communication in a world in flux. *Trends in Ecology & Evolution*, *25*, 292–300. https://doi.org/10.1016/j.tree.2009.11.003

Candolin, U. (2003). The use of multiple cues in mate choice. *Biological Reviews*, *78*, 575–595. https://doi.org/10.1017/S1464793103006158

Chaine, A. S., & Lyon, B. E. (2008). Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. *Science*, *319*, 459–462. https://doi.org/10.1126/science.1149167

Costanzo, A., Ambrosini, R., Caprioli, M., Gatti, E., Parolini, M., Canova, L., … Saino, N. (2017). Lifetime reproductive success, selection on lifespan, and multiple sexual ornaments in male European barn swallows. *Evolution*, *71*, 2457–2468.

Costanzo, A., Ambrosini, R., Caprioli, M., Gatti, E., Parolini, M., Romano, A., … Saino, N. (2017). Extrapair fertilizations vary with female traits and pair composition, besides male attractiveness, in barn swallows. *Animal Behaviour*, *134*, 183–191. https://doi.org/10.1016/j.anbehav.2017.10.019

Coyne, J. A., & Orr, H. A. (2004). *Speciation*. Sunderland, MA: Sinauer Associates.

Dor, R., Safran, R. J., Vortman, Y., Lotem, A., McGowan, A., Evans, M. R., & Lovette, I. J. (2012). Population genetics and morphological comparisons of migratory European (*Hirundo rustica rustica*) and sedentary East-Mediterranean (*Hirundo rustica transitiva*) barn swallows. *Journal of Heredity*, *103*, 55–63. https://doi.org/10.1093/jhered/esr114

Fülöp, A., Vágási, C. I., & Pap, P. L. (2017). Cohabitation with farm animals rather than breeding effort increases the infection with feather-associated bacteria in the barn swallow *Hirundo rustica*. *Journal of Avian Biology*, *48*, 1005–1014.

Klvaňa, P., Cepák, J., Munclinger, P., Michálková, R., Tomášek, O., & Albrecht, T. (2018). Around the Mediterranean: An extreme example of loop migration in a long-distance migratory passerine. *Journal of Avian Biology*, *49*, jav-01595. https://doi.org/10.1111/jav.01595

Lande, R., & Arnold, S. J. (1983). The measurement of selection on correlated characters. *Evolution*, *37*, 1210–1226. https://doi.org/10.1111/j.1558-5646.1983.tb00236.x

Maan, M. E., & Seehausen, O. (2011). Ecology, sexual selection and speciation. *Ecology Letters*, *14*, 591–602.

Maia, R., Eliason, C. M., Bitton, P.-P., Doucet, S. M., & Shawkey, M. D. (2013). pavo: An R package for the analysis, visualization and organization of spectral data. *Methods in Ecology and Evolution*, *4*, 906–913.

McGraw, K. J., Safran, R. J., & Wakamatsu, K. (2005). How feather colour reflects its melanin content. *Functional Ecology*, *19*, 816–821. https://doi.org/10.1111/j.1365-2435.2005.01032.x

Møller, A. P. (1994). *Sexual selection and the barn swallow*. Oxford, UK: Oxford University Press.

Møller, A. P., Chabi, Y., Cuervo, J. J., Lope, F., Kilpimaa, J., Kose, M., … Hirschheydt, J. (2006). An analysis of continent-wide patterns of sexual selection in a passerine bird. *Evolution*, *60*, 856–868. https://doi.org/10.1111/j.0014-3820.2006.tb01162.x

Nakagawa, S. (2004). A farewell to Bonferroni: The problems of low statistical power and publication bias. *Behavioral Ecology*, *15*, 1044–1045. https://doi.org/10.1093/beheco/arh107

Pap, P. L., Tökölyi, J., & Szép, T. (2005). Frequency and consequences of feather holes in barn swallows *Hirundo rustica*. *Ibis*, *147*, 169–175. https://doi.org/10.1111/j.1474-919x.2004.00386.x

Pap, P. L., Vincze, O., Fülöp, A., Székely-Béres, O., Pătraș, L., Pénzes, J., & Vágási, C. I. (2018). Oxidative physiology of reproduction in a passerine bird: A field experiment. *Behavioral Ecology and Sociobiology*, *72*, 18. https://doi.org/10.1007/s00265-017-2434-x

Parolini, M., Romano, A., Costanzo, A., Khoriauli, L., Santagostino, M., Nergadze, S. G., … Saino, N. (2017). Telomere length is reflected by plumage coloration and predicts seasonal reproductive success in the barn swallow. *Molecular Ecology*, *26*, 6100–6109. https://doi.org/10.1111/mec.14340

Petrželková, A., Michálková, R., Albrechtová, J., Cepák, J., Honza, M., Kreisinger, J., … Albrecht, T. (2015). Brood parasitism and quasi-parasitism in the European barn swallow *Hirundo rustica rustica*. *Behavioral Ecology and Sociobiology*, *69*, 1405–1414. https://doi.org/10.1007/s00265-015-1953-6

R Development Core Team. (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/

Reudink, M. W., McKellar, A. E., Marini, K. L. D., McArthur, S. L., Marra, P. P., & Ratcliffe, L. M. (2015). Inter-annual variation in American redstart (*Setophaga ruticilla*) plumage colour is associated with rainfall and temperature during moult: An 11-year study. *Oecologia*, *178*, 161–173. https://doi.org/10.1007/s00442-014-3167-4

Romano, A., Costanzo, A., Rubolini, D., Saino, N., & Møller, A. P. (2017). Geographical and seasonal variation in the intensity of sexual selection in the barn swallow *Hirundo rustica*: A meta-analysis. *Biological Reviews*, *92*, 1582–1600.

Romano, A., Romano, M., Caprioli, M., Costanzo, A., Parolini, M., Rubolini, D., & Saino, N. (2015). Sex allocation according to multiple sexually dimorphic traits of both parents in the barn swallow (*Hirundo rustica*). *Journal of Evolutionary Biology*, *28*, 1234–1247.

Saetre, G.-P., Moum, T., Bureš, S., Král, M., Adamjan, M., & Moreno, J. (1997). A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature*, *387*, 589–592. https://doi.org/10.1038/42451

Safran, R. J., & McGraw, K. J. (2004). Plumage coloration, not length or symmetry of tail-streamers, is a sexually selected trait in North American barn swallows. *Behavioral Ecology*, *15*, 455–461.

Safran, R. J., Vortman, Y., Jenkins, B. R., Hubbard, J. K., Wilkins, M. R., Bradley, R. J., & Lotem, A. (2016). The maintenance of phenotypic divergence through sexual selection: An experimental study in barn swallows *Hirundo rustica*. *Evolution*, *70*, 2074–2084.

Saino, N., Romano, M., Rubolini, D., Ambrosini, R., Caprioli, M., Milzani, A., … Wakamatsu, K. (2013). Viability is associated with melanin-based coloration in the barn swallow (*Hirundo rustica*). *PLoS ONE*, *8*, e60426. https://doi.org/10.1371/journal.pone.0060426

Saino, N., Romano, M., Rubolini, D., Teplitsky, C., Ambrosini, R., Caprioli, M., & Wakamatsu, K. (2013). Sexual dimorphism in melanin pigmentation, feather coloration and its heritability in the barn swallow (*Hirundo rustica*). *PLoS ONE*, *8*, e58024.

Saino, N., Szep, T., Romano, M., Rubolini, D., Spina, F., & Moller, A. P. (2004). Ecological conditions during winter predict arrival date at the breeding quarters in a trans-Saharan migratory bird. *Ecology Letters*, *7*, 21–25. https://doi.org/10.1046/j.1461-0248.2003.00553.x

Schaub, M., & Von Hirschheydt, J. (2009). Effect of current reproduction on apparent survival, breeding dispersal, and future reproduction in barn swallows assessed by multistate capture-recapture models. *Journal of Animal Ecology*, *78*, 625–635.

Scordato, E. S., & Safran, R. J. (2014). Geographic variation in sexual selection and implications for speciation in the barn swallow. *Avian Research*, *5*, 8. https://doi.org/10.1186/s40657-014-0008-4

Stinchcombe, J. R., Agrawal, A. F., Hohenlohe, P. A., Arnold, S. J., & Blows, M. W. (2008). Estimating nonlinear selection gradients using quadratic regression coefficients: Double or nothing? *Evolution*, *62*, 2435–2440. https://doi.org/10.1111/j.1558-5646.2008.00449.x

Turner, A. K. (2006). *The Barn Swallow*. London, UK: T & AD Poyser.

van Doorn, G. S., Edelaar, P., & Weissing, F. J. (2009). On the origin of species by natural and sexual selection. *Science*, *326*, 1704–1707. https://doi.org/10.1126/science.1181661

Vortman, Y., Lotem, A., Dor, R., Lovette, I., & Safran, R. J. (2013). Multiple sexual signals and behavioral reproductive isolation in a diverging population. *The American Naturalist*, *182*, 514–523. https://doi.org/10.1086/671908

Wilkins, M. R., Karaardıç, H., Vortman, Y., Parchman, T. L., Albrecht, T., Petrželková, A., … Safran, R. J. (2016). Phenotypic differentiation is associated with divergent sexual selection among closely related barn swallow populations. *Journal of Evolutionary Biology*, *29*, 2410–2421. https://doi.org/10.1111/jeb.12965

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Supporting information

Table S1. Partial quadratic selection differentials (gradients, controlling for the effect of wing length and tarsus length) for the two putative sexual traits measured in first-time breeders and experienced male and female barn swallows from the Czech (CZ) and Romanian (RO) populations.

	CZ				
	N	*γ* (SE)	*t*	*P*	*P adj*
Male					
Tail length					
First-time breeders	134	0.003 (0.037)	0.07	0.9439	0.9439
Experienced birds	156	0.051 (0.053)	0.96	0.3389	0.4983
Brightness					
First-time breeders	134	0.014 (0.037)	0.39	0.7011	0.9678
Experienced birds	156	–0.007 (0.046)	–0.16	0.8740	0.8740
Female					
Tail length					
First-time breeders	161	0.013 (0.030)	0.44	0.6635	0.8211
Experienced birds	150	–0.036(0.064)	–0.57	0.5720	0.5980
Brightness					
First-time breeders	161	–0.026 (0.029)	–0.93	0.3549	0.5323
Experienced birds	150	–0.031 (0.054)	–0.58	0.5658	0.8487

	RO				
	N	*γ* (SE)	*t*	*P*	*P adj*
Male					
Tail length					
First-time breeders	138	0.007 (0.036)	0.18	0.8537	0.8537
Experienced birds	159	0.057 (0.027)	2.14	0.0341	0.1024
Brightness					
First-time breeders	17	0.215 (0.255)	0.84	0.4284	0.6597
Experienced birds	17	–0.036 (0.083)	–0.43	0.6786	0.7564
Female					
Tail length					
First-time breeders	178	–0.072 (0.032)	–2.29	0.0230	0.0691
Experienced birds	128	0.017 (0.023)	0.75	0.4543	0.6020
Brightness					
First-time breeders	14	0.290 (0.086)	3.37	0.0280	0.0652
Experienced birds	16	–0.284(0.209)	–1.36	0.2227	0.6207

PUBLIKACE 4

Michálková R., Tomášek O., Adámková M., Kreisinger J. & Albrecht T. (2019). **Extra-pair paternity patterns in European barn swallows *Hirundo rustica* are best explained by male and female age rather than male ornamentation.** *Behavioral Ecology and Sociobiology* 73: 119

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ORIGINAL ARTICLE

Extra-pair paternity patterns in European barn swallows *Hirundo rustica* are best explained by male and female age rather than male ornamentation

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Abstract

Adaptive explanations for the evolution of extra-pair paternity (EPP) in birds often assume cuckolding males to be better-ornamented than cuckolded males. Several studies have confirmed that either male sexual ornamentation is associated with EPP or that phenotypes of cuckolded and cuckolding males differ. Expression of male ornamentation may change with age; however, a recent meta-analysis has identified age itself as an important factor that differed in cuckolding and cuckolded males. The age of social female partner may also affect EPP, though this has received little attention. Here, by using detailed data on age of individual barn swallows *(Hirundo rustica rustica*), we identified age as the major predictor of male and female promiscuity. Our results revealed that, whereas a male's ability to obtain an extra-pair mate increased linearly with age, the only predictor of the probability of a male being cuckolded was the age of his social partner, with older females engaging more frequently in EPP. In contrast, male ornamentation was not significantly related to EPP pattern. Tarsus length was the sole significant phenotypic trait in comparison between cuckolding and cuckolded males. Our data provide little support for the hypothesis that extra-pair mate choice in our barn swallow population was ornament driven. This may indicate either a non-adaptive scenario for EPP, for example with older males better able to coerce females into copulation, or EPP mating based on other than absolute mate-choice criteria associated with the expression of male ornamentation.

Significance statement

We analysed patterns of extra-pair paternity (EPP) in barn swallows. Derived from observation of 160 nests, our results appear to differ from the findings of some previous studies that identified ornamental traits as being associated with extra-pair and within-pair paternity (WPP) in this iconic model taxon of sexual selection. In particular, tail streamer length had no detectable association with WPP or a male's ability to obtain an extra-pair partner. Pairwise comparisons of cuckolded and cuckolding males, involving 76 mixed paternity nests, also supported the hypothesis that male ornamentation does not play a role in determining EPP patterns in the focal barn swallow population. When statistically controlled for clutch initiation date, the probability of obtaining an extra-pair partner only increased with increasing age of males, while WPP was only associated with the age of their female social partners. Our data provide little support for the hypothesis that extra-pair mate choice in our barn swallow population is ornament driven and indicated that age, rather than ornaments, would be a better predictor of paternity.

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Keywords Extra-pair fertilisations · Within-pair paternity · EPP · Sexual ornamentation · Sexual selection · Tail streamer length

Introduction

Female mate choice could be pre-copulatory (social mate choice) or post-copulatory (sire choice), via sexual promiscuity and subsequent sperm competition or cryptic female choice

(Birkhead 2010). Over recent decades, female promiscuity or multiple mating has been identified in many taxa, including mammals (Bryja et al. 2008), reptiles (Uller and Olsson 2008) and fishes (Coleman and Jones 2011); however, the major research focus has been on extra-pair paternity (EPP) in socially monogamous birds (Griffith et al. 2002). The drivers behind the evolution of promiscuous behaviour in birds remain unclear, despite intense research over the last three decades. While male interest in attending extra-pair copulations (EPC) appears to be intuitive (Forstmeier et al. 2014), female motivation to engage in EPC is much less obvious. Both adaptive and non-adaptive hypotheses have been proposed to explain female pursuit behaviour (Forstmeier et al. 2014). Adaptive hypotheses state that females benefit from EPC with major attention being devoted to genetic benefits in form of good genes or higher offspring heterozygosity through mating with higher-quality (i.e. more ornamented) and/or more experienced (older) extra-pair males (Kempenaers et al. 1992; Mays et al. 2008; Forstmeier et al. 2014; Lyu et al. 2018). However, the empirical evidence for such benefits remains equivocal and controversial (Arnqvist and Kirkpatrick 2005; Albrecht et al. 2006; Akçay and Roughgarden 2007; Eliassen and Kokko 2008). This has led to the emergence of non-adaptive models that assume that female promiscuity may evolve and be maintained as a by-product of selection on other traits, even if there is no direct or indirect fitness benefits to females (reviewed in Forstmeier et al. 2014; Hsu et al. 2015; Lyu et al. 2018).

Where sexual promiscuity occurs in socially monogamous birds, male fitness may be determined by the ability to sire offspring and protect paternity in his own nest (within-pair fertilisation) and/or the ability to sire offspring in nests of other males in the population (extra-pair fertilisation; Webster et al. 1995). Within-population studies have identified a range of phenotypic traits associated with male within-pair and extra-pair fertilisation success, such as plumage colouration, morphological traits or body size (e.g. Griffith et al. 2002; Bitton et al. 2007). Higher fertilisation success in more ornamented males could be interpreted as resulting from female extra-pair mate choice. This type of evidence, however, is often confounded by other variables that are often overlooked in paternity studies. As an example, expression of many phenotypic traits, including male ornamentation, increases with age (Freeman-Gallant et al. 2010) and it has become apparent that some of the reported correlative relationships between male EPP success and male ornamentation could be confounded by the effect of male age (Freeman-Gallant et al. 2010; Lifjeld et al. 2011).

In general, male reproductive success increases with age, but declines later in life due to senescence (e.g. Mauck et al. 2004; Froy et al. 2013; Hsu et al. 2017). Age itself may also be associated with male experience and body condition and, as such, may be targeted by female choice (Manning 1985; Kokko 1998). At the same time, age could be associated with individual male experience, while both the age and body size could be associated with his ability to force females to engage in extra-pair mating, or his success in male-male competition over access to fertile females (Westneat and Stewart 2003; Hsu et al. 2015). Using available data, recent meta-analyses (Akçay and Roughgarden 2007; Hsu et al. 2015) have shown that male age, but not male ornamentation, was the main trait that differed in cuckolded and cuckolding males across bird populations. This appears to contradict adaptive models explaining the evolution of EPC in birds as driven by indirect benefits (i.e. genetic compatibility or good genes; Kempenaers 2007) that increase female fitness and open space for alternative non-adaptive explanations (i.e. male manipulation hypothesis proposing that older and larger males may be better at convincing or forcing females to copulate; see also Forstmeier et al. 2014; Hsu et al. 2015).

EPP research typically focuses on male traits, yet several studies have suggested that female characteristics may also influence variation in EPP (e.g. Whittingham and Dunn 2010; Grunst and Grunst 2014; Moreno et al. 2015; Costanzo et al. 2017a). The relationship between female age and EPP has received little attention, however, and the results of the few relevant studies (Stutchbury et al. 1997; Ramos et al. 2014; Moreno et al. 2015; Costanzo et al. 2017a) are controversial. In addition, pair age compatibility could also affect the incidence of cuckoldry in avian nests as, for example, older females paired with young males could be better able to avoid mate guarding and engage in EPC (Dietrich et al. 2004; Bouwman and Komdeur 2005; Ramos et al. 2014).

Here, we used data from an intensively studied promiscuous European barn swallow, *Hirundo rustica* population in South Bohemia (Czech Republic; Petrželková et al. 2015; Kreisinger et al. 2015; Vitousek et al. 2016), to determine traits associated with within-pair and extra-pair mating. Barn swallows are small, socially monogamous migratory passerines commonly used in studies of sexual selection (e.g. Møller 1994; Saino et al. 1997; Safran and McGraw 2004; Vortman et al. 2011; Hasegawa et al. 2012). A range of ornamental traits has been identified as being associated with social mate choice in barn swallow populations. These include (1) *tail streamer length* (Møller 1994; Saino et al. 1997), (2) *ventral colouration* (Safran and McGraw 2004; Safran et al. 2005), (3) *white tail spot size and shape* (Kose et al. 1999; Hasegawa et al. 2010; Møller 2017) and (4) *throat patch colouration and size* (Ninni 2003; Safran et al. 2005; Hasegawa et al. 2010; Hasegawa et al. 2012). In contrast to social mating, the role of similar traits in extra-pair mating is less well documented (but see Saino et al. 1997; Kleven et al. 2006a; Lifjeld et al. 2011; Romano et al. 2017). While a number of studies have documented an impact of age on social and/or extra-pair mating in this species (Møller et al. 2003; Lifjeld et al. 2011; Vortman

et al. 2011; Costanzo et al. 2017a), males were usually dichotomously classified as 'yearlings' or 'older', preventing a detailed analysis of associations between age and male performance in EPP.

As detailed data on individual age were available in our study population, it was possible to separate effects of male ornamentation and age (using both linear and second-order polynomial effects to evaluate potential senescence in male performance; Balbontín et al. 2007) and evaluate how the age of a male's social mate affected the probability of cuckoldry. The main objectives of this study were as follows: (1) to evaluate the effect of male age and ornamentation on two potential components of male fitness, i.e. within-pair and extra-pair fertilisation success (e.g. Webster et al. 1995; Albrecht et al. 2007; Webster et al. 2007), with the possibility that social female age may be associated with variation in WPP; (2) to directly compare phenotypes of cuckolding and cuckolded males, focusing on ornamentation, age and tarsus length (a proxy for male structural size; Kempenaers et al. 1997). Body size (represented by tarsus length, as this morphological trait is related to overall structural body size in passerines; Kempenaers et al. 1997; Kruuk et al. 2001) is considered in this study because previous studies have indicated that male body size could actually be associated with male ability to coerce females to copulation, or with male ability to get access to the female through male-male interactions (Hsu et al. 2015). Female age is considered, as it is associated with female promiscuity in some passerine species (e.g. Kempenaers et al. 1999).

Methods

Study area and general field procedure

The field study was carried out from 2010 to 2013 at two isolated farms in the Třeboňsko Protected Landscape Area (Czech Republic) separated by approximately 10 km: Šaloun near Lomnice nad Lužnicí (49° 4′ 7.762″ N, 14° 42′ 36.521″ E) and Hamr near Lužnice (49° 3′ 25.288″ N, 14° 46′ 10.82″ E). Both localities are similar with respect to the number of breeding pairs (average 20 breeding pairs per locality and year) and rate of EPP (chi-squared test, $\chi^2 = 1.13$, df = 1, $p = 0.332$). Most of the birds breed in colonies in the same barn, with just a few solitary breeding pairs partly isolated from the other nests (max 100 m from the main colony) but always interacted with birds from the whole colony. Adults were systematically captured with mist nets on several occasions during the breeding season. We captured the vast majority of the adults every year, and almost all social parents were identified by individual combinations of colour rings. Similarly, we have only very few observations of unringed birds (less than 5% of individuals) in our study sites each season by the end of June. Each individual was marked with an aluminium ring (National Museum of Prague) and a unique combination of coloured plastic rings (AVINET) that allowed identification in the field. Social pairs were assigned by observing nest defence and repeated feeding of nestlings (or incubating eggs in the case of females). Each adult bird was measured for morphological variables (tarsus length and tail streamer length) at the time of first capture. Feather samples were collected from the throat and ventral plumage (more than ten feathers per region) for subsequent colour analysis, and photographs (with a scale) were taken of the white spots on the tail. We also sampled a small amount of blood (~20 μL) by brachial venepuncture for parentage analysis. All nests were found during the egg-laying period and subsequently checked at three-day intervals. Clutch initiation date was estimated by observing the appearance of the first egg laid in the nest or was deduced based on the assumption that females laid one egg each day. Nestlings were ringed at the age of 9 days and a blood sample taken from the brachial vein. All blood samples were stored in 96% ethanol at − 20 °C until DNA extraction.

Between 2010 and 2013, barn swallows initiated 160 first breeding attempts on our study plot. We used first seasonal breeding attempts only as not all pairs raise two broods per year and the intensity of sexual selection is higher during the start of the breeding season (Romano et al. 2017). For pairwise comparisons, we included 76 nests with mixed paternity and compared the cuckolded (social) male with the corresponding cuckolding (extra-pair) male/males directly. In this analysis, we also included 18 nests with social males cuckolded by extra-pair males whose nests were inaccessible and/or clutch initiation date could not be precisely assessed. In the remaining 58 nests, we were able to compare clutch initiation date between cuckolded and cuckolding males. We knew the exact age of birds if they were ringed as nestlings in the study area (39 males and 24 females from the main dataset of 160 nesting events). Ringing of both nestlings and adults started in 2008, but intense and systematic capturing and sampling of both nestlings and adults started in 2010. We therefore decided to include only birds with known exact age in 2010 (13 males sampled as nestling in 2008 or 2009).

Because every year (2010–2013) we captured and marked the vast majority of breeding adults and because barn swallows show extremely high breeding philopatry (adults rarely move to a different colony to breed in consecutive year; also see Møller 1994; Møller et al. 2003; Saino et al. 2013; Costanzo et al. 2017b), individuals that were captured without rings and were not captured as adults in the previous year were assumed to be 1-year-old birds originating from elsewhere (see Møller 1994; Møller et al. 2003; Costanzo et al. 2017b). Although these estimates represent minimum age, they likely equal to actual age in almost all the individuals owing to extremely high breeding philopatry and intense capture effort.

We included these individuals into the analysis as many bred in multiple years, thereby allowing us to use these birds in the assessment of age-related effects (Bowers et al. 2015). Overall, barn swallow age ranged between 1 and 5 years. In the analyses, we used only four age classes as 5-year-old individuals were rare in our dataset (two males and one female) and we assigned these 5-year-old individuals age ≥ 4 to prevent them being influential points and affect the results. In our study area, breeding colonies were settled in isolated farms (min. 1 km distance from the nearest human settlement or barn and min. 10 km from the nearest breeding colony); thus, it is unlikely that males were siring offspring in neighbouring colonies. This was partly confirmed by analysis of paternity, which indicated that males from neighbouring sites do not sire offspring in our breeding colonies.

Measurement of tail spot area and colour analysis

Photographs of the white spots on the five tail feathers from the right side of the body were taken using a Nikon D40 digital camera with a millimetre scale. The area of the white tail spots was analysed from the photographs with ImageJ software, using the freehand selection function to encircle each spot. Tail white spot area was expressed for each individual as the total area of all spots on right tail feathers. This estimate is highly correlated with the size of white spot on the outermost right feather in our population (RM et al. unpublished data; Pearson correlation coefficient, $r = 0.83$, $n = 272$ males, $p < 0.001$).

Feather colouration was analysed using an AvaSpec 2048 reflectance spectrometer with an AvaLight-XE light source (Avantes, Netherlands). The spectrometer sensing-probe was equipped with a metal adapter that shielded the measured area from ambient light and held the probe at a constant distance of 3.5 mm above the sample. At least ten feathers were arranged on a white paper index card in order to achieve a layer equivalent to the actual ordering of feathers on the body. Each sample was measured three times at the distal part of the feather with the probe held perpendicular. The spectrometer was calibrated against a WS-2 white standard (Avantes, Netherlands) and absolute dark after measuring eight samples. Reflectance data were analysed using the R software v. 3.1.2 (R Core Team 2014) and the pavo package (Maia et al. 2013). The three measurements from each sample were averaged and smoothed by a span of 0.25. Subsequently, the colour was analysed using avian visual model based on relative stimulation of four photoreceptor types followed by projection to the tetrahedral colour space—a method that provides the most biologically relevant quantification of how colour is perceived by a receiver (Goldsmith 1990; Endler and Mielke 2005; Stoddard and Prum 2008). The spectral sensitivity of blue tit (*Cyanistes caeruleus*) implemented in the pavo package was adopted for visual modelling (Costanzo et al. 2017b) with standard daylight (D65) being used as an illuminant. Modelling in the avian tetrahedral colour space produces three colour metrics representing a hue component expressing relative stimulation of red, green and blue retinal cones (θ), a UV hue component (φ) and saturation (achieved chroma; *rA*). The repeatability, estimated by repeated measurements of the same feather samples ($n = 20$), was as follows: throat θ repeatability = 0.79 ($F_{19,20} = 8.73$, $p < 0.001$), ventral θ repeatability = 0.67 ($F_{19,20} = 5.03$, $p < 0.001$), throat φ repeatability = 0.72 ($F_{19,20} = 6.04$, $p < 0.001$), ventral φ repeatability = 0.88 ($F_{19,20} = 15.05$, $p < 0.001$), throat *rA* repeatability = 0.82 ($F_{19,20} = 9.88$, $p < 0.001$), ventral *rA* repeatability = 0.88 ($F_{19,20} = 5.82$, $p < 0.001$). Previous analysis has indicated that all three metrics are heritable in ventral plumage of barn swallows (Hubbard et al. 2015). We only used ventral θ in our study, because all three metrics were highly intercorrelated in ventral region (Pearson correlation coefficients, $n = 266$; θ vs. φ: $r = 0.86$, $p < 0.001$; θ vs. *rA*: $r = 0.96$, $p < 0.001$; φ vs. *rA*: $r = 0.83$, $p < 0.001$). By contrast, we used both throat θ and φ, as they were largely independent of each other ($r = -0.11$, $p = 0.03$), while both were moderately correlated to *rA* (θ: $r = 0.60$, $p < 0.001$; φ: $r = 0.65$, $p < 0.001$). All ornament measures were performed blind with respect to the outcome of parentage assignment and/or knowledge on individual age.

Parentage assignment

DNA was extracted from blood and tissue samples using the DNeasy® Blood & Tissue kit (Qiagen). All individuals were genotyped at six highly polymorphic microsatellite autosomal loci previously developed for barn swallows (Online Resource Table S1). The combined exclusion probability for the marker set was higher than 0.9999. Significance of parentage assignment was assessed using the observed Delta statistics value with the strict 95% confidence criterion selected. Critical delta values were computed using 10 000 simulations, based on the distribution of allele frequency and estimated genotyping errors 1% observed in our population. We assumed that 80% of breeding females and 90% of breeding males were sampled in the simulations. According to the simulations, the probability of non-assignment of the mother alone, father alone or parent-pair was 4%, 2% and 1%, respectively. A detailed description of the methods (PCR conditions, genotype scoring, binning and genotyping errors) is provided in Petrželková et al. (2015).

Parentage assignment was undertaken using Cervus version 3.0.3 (Kalinowski et al. 2007) and Colony software version 2.0 (Wang 2004; Jones and Wang 2010). As brood parasitism and quasi-parasitism was previously detected in our study population (Petrželková et al. 2015), we accounted for this in the parentage analysis. To undertake paternity exclusions, therefore, we defined a nestling with more than one mismatch with social male/female as an extra-pair/parasitic

young in order to avoid false exclusions caused by null alleles at single loci (Dakin and Avise 2004). Detailed methods related to paternity and maternity assignment are presented elsewhere (Petrželková et al. 2015). Using these methods, we successfully obtained genotypes of all males and females and 694 young from 160 nests. Genetic fathers were identified for 688 young (99.1%).

Statistical analysis

All statistical analyses were undertaken using the R software package v. 3.4.0 (R Core Team 2017). We evaluated the effect of explanatory variables (tarsus length, tail streamer length, throat θ and φ, ventral θ, area of white tail spots, clutch initiation date, linear and second-order polynomial effect of male and female age—as continuous variables) on the male's ability to obtain an extra-pair partner and the probability of paternity loss. Within-pair and extra-pair success were coded as binary dependent variables in this analysis (i.e. 0 = no extra-pair offspring sired outside the nest or no extra-pair offspring detected in the male's nest, respectively; 1 = at least one extra-pair offspring sired or at least one extra-pair offspring detected in the male's nest, respectively). As extra-pair offspring tend to be detected more often in larger broods, brood size was included as a covariate when analysing WPP. We also evaluated male age x female age interaction, as age incompatibility may also play a role in WPP patterns (Dietrich et al. 2004; Bouwman and Komdeur 2005; Ramos et al. 2014). In parallel, we also modelled male extra-pair fertilisation success in relation to the same set of predictors, with the number of extra-pair offspring sired as a dependent variable. Similarly, we ran models with the proportion of extra-pair and within-pair offspring detected in the nest as dependent variables to further explore patterns in WPP. The results of these analyses were qualitatively and quantitatively similar to those using binary dependent variables (reported in the Online Resource). As number of males (n = 33) and females (n = 35) provided more than one data point in the data set (range 2–4), we used the glmer function in the lme4 package for R (Bates et al. 2015) to fit generalised linear mixed-effects models with male and associated social female identity and year-colony (with 8 levels) included as random effects, with logit (binomial) or log (Poisson) link functions based on the nature of the dependent variable (binary, proportion or count, respectively). Continuous explanatory variables were standardised (z-transformed) before analysis to improve the convergence of complex initial models. Clutch initiation date was centred within each year to account for differences in the timing of breeding between years. To obtain p values, we performed likelihood ratio tests comparing models with and without specific fixed effects. Final models were identified through backward elimination of non-significant effects ($p > 0.05$) based on the drop1 function in R and associated changes in deviance expressed as χ^2. We estimated the variance explained by the fixed effects of our mixed-effects models as marginal R^2-values, using the r.squaredGLMM function within the MuMIn package for R (Bartoń 2016). To estimate potential multicollinearity among model predictors, variance inflation factor (VIF) was calculated for each predictor in the full model using the vif.mer function (see Online Resource, function 1). VIF was never > 1.48, indicating only moderate levels of collinearity among predictors (Zuur et al. 2010). We estimated dispersion parameters to check for overdispersion in those models with proportion and count as dependent variables. In all models evaluated, the dispersion parameter was always close to 1.0 (range 0.89–1.02). We also calculated repeatability in male propensity to be cuckolded or his ability to sire at least one extra-pair offspring using the rptR package for R (number of bootstraps set to 1000; Stoffel et al. 2017). Pairwise t tests were used to identify traits that differed between cuckolded and cuckolding males. In cases when more than one extra-pair male was cuckolding in the nest of a single social male, we used the mean values of the extra-pair fathers. Unless stated otherwise, means are presented together with their standard errors (SE).

Results

Frequency of extra-pair paternity

EPP was detected in 51.2% of nests (82/160), with 19.8% (138/694) of offspring being sired by an extra-pair male (Online Resource Table S2). There was no difference between years in the proportion of EPP young in the population (chi-squared test, $\chi^2 = 5.80$, df = 3, $n = 160$, $p = 0.12$). The number of extra-pair young in nests with mixed paternity varied from one to five (median = 1.50; mean ± SE = 1.70 ± 0.09). The number of extra-pair fathers detected in nests containing extra-pair young varied between one and three (median = 1.00; mean ± SE = 1.22 ± 0.05). The number of extra-pair offspring sired by males successful in extra-pair mating varied from one to six (median = 2.00; mean ± SE = 2.13 ± 0.15).

Older males tended to have older females as social partners ($\chi^2 = 28.67$, Δdf = 1, $p < 0.05$). While this potentially indicates assortative mating by age, it was most probably driven by clutch initiation date as both older males and older females initiated their clutch earlier (Online Resource Table S3).

Factors affecting male success in extra-pair fertilisations

There was no association between a male extra-pair fertilization success and propensity of being cuckolded, the frequency of males able to obtain EPP being similar in those that lost

paternity and those that sired all offspring in their own nest (chi-square test, $\chi^2 = 2.03$, df = 1, $p = 0.15$). In our initial analysis evaluating the effect of male age (linear or second-order polynomial) on ornamental traits, only tail streamer length and area of white tail spots (but not feather colouration) significantly increased linearly with male age (Online Resource Table S4). There was no detectable evidence for senescence in our data set and range of male ages available, but it should be noted that sample size was low for this kind of analysis with only few old individuals. We further evaluated the effect of male characteristics on ability to sire at least one extra-pair offspring. A set of univariate models (Online Resource Table S5) indicated male age as the most important predictor of ability to engage in EPP, followed by clutch initiation date. There was no detectable evidence for any second-order polynomial effect of male age on ability to succeed in EPP (comparison of linear and second-order polynomial effect of male age: $\chi^2 = 1.86$, $\Delta df = 1$, $p = 0.17$). In the full model, male EPP success was positively associated with age (linear effect; Fig. 1), with a tendency for earlier breeding males to engage in EPP more frequently, independent of male age (Table 1). The final model included linear effect of male age only (slope ± SE = 0.96 ± 0.20; marginal pseudo $R^2 = 0.22$; $\chi^2 = 28.44$, $\Delta df = 1$, $p < 0.001$). In a set of univariate models for the number of extra-pair offspring sired by males, linear effect of male age was again the most important sole predictor, followed by clutch initiation date of male social nest and tarsus length (Online Resource Table S6). There was no association between male age and the number of extra-pair young sired out when analysing only those males succeeding in siring at least one extra-pair offspring ($\chi^2 = 0.69$, $\Delta df = 1$, $p = 0.40$), indicating that a male's age affects his ability to obtain at least one extra-pair partner but not the actual number of extra-pair offspring sired. Repeatability in male extra-pair siring success (binary response variable) over successive years was negligible (repeatability = 0.0, 95%CI = 0–0.22, $n = 33$ males with repeated observations).

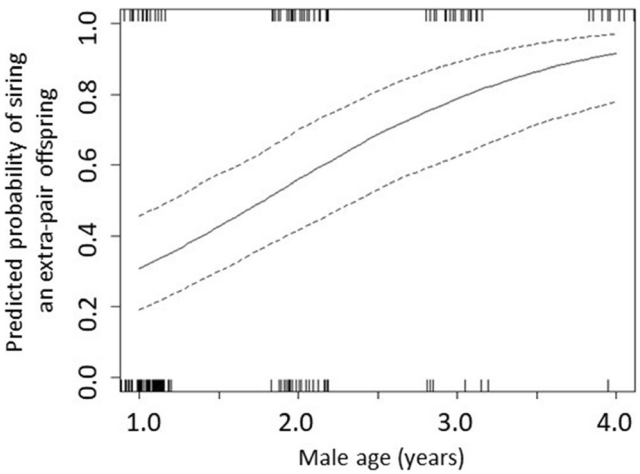

Fig. 1 Predicted probability of males siring extra-pair offspring in relation to male age. Dashed lines denote 95% confidence intervals around the predicted values based on a generalised linear mixed-effects model (binary response variable and logit link function), with male identity treated as a random grouping variable. See text for further details

Table 1 Male extra-pair fertilisation success in relation to male phenotype, male age and timing of breeding ($n = 160$ breeding attempts). Estimates are based on a generalised linear mixed model (male identity and year-locality as random grouping factors) with binary response variables (1 = at least one offspring sired extra-pair, 0 = no offspring sired extra-pair) and logit link function. Predictors were standardised (z-transformed) prior to analysis. Explanatory variable significantly associated with male extra-pair fertilisation success after simplification of the full model (see main text for further details) is indicated in italics

	Estimate	Std. error	*z*	*p*
Intercept	0.049	0.299	0.166	0.868
Tail streamer length	0.079	0.197	0.405	0.685
Tail spots	0.156	0.207	0.756	0.449
Ventral θ	0.025	0.201	0.123	0.902
Throat θ	0.141	0.260	0.543	0.587
Throat φ	0.117	0.253	0.463	0.643
Tarsus length	0.023	0.187	0.127	0.899
Clutch initiation date	−0.368	0.219	−1.684	0.092
Male age	*0.829*	*0.240*	*3.453*	*< 0.001*

Factors affecting male paternity loss

There was no detectable evidence for a non-linear effect of male and female age on the probability of WPP loss (comparison of linear and second-order polynomial effect of age; males: $\chi^2 = 0.07$, $\Delta df = 1$, $p = 0.78$; females: $\chi^2 = 1.07$, $\Delta df = 1$, $p = 0.30$). Univariate models indicated linear female age as the sole predictor explaining the occurrence of EPP in nests (Online Resource Table S7). In the full model utilising the full set of predictors in addition to male and female age (linear) and male age x female age interaction, interaction term was not significant in explaining patterns of WPP loss ($\chi^2 = 0.01$, $\Delta df = 1$, $p = 0.91$) and, as such, was removed from the model. In the reduced final model (Table 2), female age only was again associated with the probability of her social partner being cuckolded. Thus, the final model for occurrence of EPP in nests included female age only (slope ± SE = 0.51 ± 0.19; marginal pseudo $R^2 = 0.07$; $\chi^2 = 8.46$, $\Delta df = 1$, $p < 0.05$; brood size included as a covariate in the model). Extra-pair offspring occurred more often in nests of

Table 2 Male within-pair fertilisation success in relation to male phenotype, male and social partner age and timing of breeding (brood size treated as a covariate; $n = 160$ breeding attempts). Estimates are based on a generalised linear mixed model (male and female identity and year-locality as random grouping factors) with binary response variables (1 = at least one extra pair offspring occurred in the male's nest, 0 = no extra-pair offspring detected in the male's nest) and logit link function. Predictors were standardised (z-transformed) prior to analysis. Explanatory variable significantly associated with male within-pair fertilisation success after simplification of the full model (see main text for further details) is indicated in italics

	Estimate	Std. error	z	p
Intercept	0.063	0.246	0.255	0.798
Tail streamer length	0.119	0.196	0.608	0.543
Tail spots	0.237	0.196	1.211	0.226
Ventral θ	0.123	0.204	0.601	0.548
Throat θ	−0.170	0.253	−0.672	0.502
Throat φ	−0.172	0.252	−0.680	0.496
Tarsus length	0.162	0.187	0.870	0.384
Clutch initiation date	0.026	0.215	0.123	0.902
Brood size	0.363	0.197	1.837	0.066
Male age	−0.104	0.228	−0.456	0.649
Female age	*0.566*	*0.238*	*2.379*	*0.017*

Table 3 Pairwise comparison of cuckolding and cuckolded males. Estimates are based on pairwise t tests ($n = 76$ nests, [a] $n = 58$ nests). Cuckolding males as a reference group. Variable significantly differing between cuckolded and cuckolding males is indicated in italics

	Mean Diff.	Std. error	t	df	p
Tail streamer length	1.031	1.707	0.604	75	0.547
Tail spots	12.922	11.782	1.097	75	0.276
Ventral θ	−0.002	0.008	−0.219	75	0.826
Throat θ	0.001	0.005	0.211	75	0.834
Throat φ	−0.005	0.074	−0.562	75	0.575
Tarsus length	*−0.218*	*0.083*	*−2.608*	*75*	*0.011*
Clutch initiation date[a]	0.247	0.158	1.556	57	0.125
Male age	−0.211	0.167	−1.256	75	0.213

older females than younger females (Fig. 2), regardless of male age. In a set of univariate models with the proportion of within-pair and extra-pair offspring in the nest as the dependent variable, linear effect of female age was again the most important predictor (Online Resource Table S8). Again, there was no association between female age and the proportion of extra-pair young in the nest when the analysis focused on nests with at least one extra-pair young detected ($\chi^2 = 0.58$, $\Delta df = 1$, $p = 0.44$). Repeatability in male propensity to be cuckolded (binary response variable) was low (repeatability = 0.06, 95%CI = 0–0.31, $n = 33$ males with repeated observations).

Comparison between extra-pair sires and cuckolded males

A comparison of cuckolded and cuckolding males identified only tarsus length distinguishing between them (cuckolding males had longer tarsi than males they cuckolded; Table 3).

Discussion

In this study, we examined patterns of WPP and EPP in relation to a set of male ornamentation, male age and age of social partner in a Central European barn swallow population. The main findings of the present study are a linear increase in the male's ability to obtain extra-pair mating with age, a linear increase in the probability of being cuckolded with age of social female and no detectable effect of male ornamentation on EPP. We found no detectable relationship between male within-pair and extra-pair fertilisation success, implying that EPP in barn swallows may not contribute substantially to variation in male fitness (see also Albrecht et al. 2007; Webster

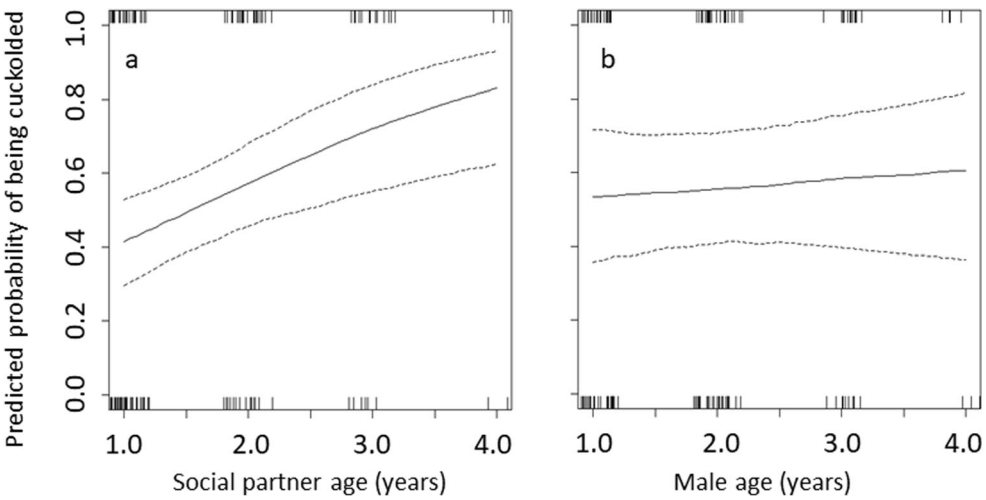

Fig. 2 Variation in predicted probability of being cuckolded in relation to (**a**) female age and (**b**) male age. Dashed lines denote 95% confidence intervals around the predicted values based on a generalised linear mixed-effects model (binary response variable and logit link function), with male and female identity treated as random grouping variables. See text for further details

et al. 2007). Our finding of negligible repeatability in a male's ability to sire extra-pair offspring or avoid paternity loss in his nest may also indicate low contribution of EPP to male (lifetime) fitness in our barn swallow population.

An increase in total reproductive success or extra-pair and within-pair fertilization success with male age, with a later decline due to senescence, has recently been demonstrated in several passerine species (reviewed in Hsu et al. 2017), including barn swallows (e.g. Møller and de Lope 1999). Several studies have documented the impact of age on paternity in barn swallows, but with the following limitations: (1) age was dichotomously classified as 'yearlings' or 'older' (Lifjeld et al. 2011; Vortman et al. 2011; Costanzo et al. 2017a), preventing an evaluation of the effect of actual age and the potential effect of senescence; (2) sample size has often been limited (Lifjeld et al. (2011) - *H. r. erythrogaster*, yearlings $n = 18$ versus older $n = 38$; Vortman et al. (2011) - *H. r. transitiva*, yearlings $n = 6$ versus older $n = 10$); (3) studies have not considered EPP outside the nest in male reproductive success (Møller and de Lope 1999; Møller et al. 2009a) or (4) studies have focused primarily on the effect of ornamentation, arrival date and body condition controlling for age and have not analysed the direct effect of age on components of reproductive success (Møller et al. 2003, 2009a). Moreover, to our knowledge, few studies have evaluated female age in relation to WPP in barn swallows (Møller et al. 2009a; Costanzo et al. 2017a), although Balbontín et al. (2007, 2012) analysed female age in relation to annual fecundity and clutch size. In this study, we were able to statistically control for age effects due to detailed data on individual age in the study population and detailed parentage assignment. Our results revealed that linear effect of age only (not polynomial) had a statistically significant effect on patterns of both WPP and EPP. We observed no decline in reproductive success as birds became older, indicating no detectable senescence. It should be stressed, however, that we only studied reproductive success in barn swallows over four seasons and we assigned a few 5-year-old individuals age four to prevent them being influential points and affect the results. A decline in reproductive performance may occur later in life (Hsu et al. 2017). On the other hand, long-term studies of European barn swallows have shown a decline in reproductive success and sperm performance after just 3 years (Møller and de Lope 1999; Balbontín et al. 2007; Møller et al. 2009b). In addition, our estimation of age was only indirect (also see Costanzo et al. 2017a), and we cannot exclude the possibility that some birds estimated as second-year individuals (first time breeders) were actually older. However, this should not affect our main result, i.e. an increase of male extra-pair success with age. In fact, if our second-year male group also contains some older individuals, our estimates of age effects on EPP would be conservative and real effects possibly higher than reported in our study.

The effect of male age on the ability to sire extra-pair offspring could be interpreted as a combination of female preference for higher-quality, older males and/or male behavioural strategies that vary with age (Brooks and Kemp 2001; Westneat and Stewart 2003; Kleven et al. 2006b). The former mechanism would require an ability of females to assess the age of males. Age could be reflected by ornamentation, such as tail streamer length or colouration (Costanzo et al. 2017b); however, ornamentation alone did not explain male performance in our study. Males that survive to old age could also be of higher genetic quality (Richardson and Burke 1999; Brooks and Kemp 2001) as they have managed to avoid predators and diseases; hence, they may be healthier, and their vigour could be preferred by females, independent of ornamentation. Alternatively, older males may be more experienced or aggressive and, consequently, better able to coerce females into mating with them or succeed in male-male competition for mates (Westneat and Stewart 2003), as predicted by the 'male manipulation hypothesis' (Hsu et al. 2015). In our study, while we were unable to distinguish between these scenarios, age was a better predictor of male EPP success than ornamental traits. Moreover, tarsus length was greater in cuckolding males than cuckolded males, supporting the findings of some previous studies (e.g. Foerster et al. 2003; Canal et al. 2011). Tarsus length appears to be related to overall structural body size in passerines (Kempenaers et al. 1997; Kruuk et al. 2001) and this finding may further support the idea that a male's ability to convince or coerce females to mate contributed to EPP pattern in our population (Hsu et al. 2015). Interestingly, comparisons between cuckolded and cuckolding males have shown no significant difference in plumage ornament or age, in contrast to previous studies on other passerines (e.g. Foerster et al. 2003; Bouwman et al. 2007; Hsu et al. 2015; Edme et al. 2016; but see Bitton et al. 2007).

Another important predictor of male extra-pair success could be the timing of breeding, as there is a positive correlation between clutch initiation date and male/female age, and early breeding may increase the number of available extra-pair partners. In our study, males that started their clutch earlier had a higher probability of successful EPP and sired more extra-pair young in other nests. While early-breeding males may not be more effective at mate guarding in terms of loss of paternity in their own nest, they may be better able to allocate time to seeking extra-pair mates (O'Brien and Dawson 2011). In our population, while time of breeding was correlated with both male and female age (both older males and older females initiated their clutches earlier), age was a better predictor of a male's ability to obtain an extra-pair partner than clutch

initiation date itself. In comparison, paternity loss was not affected by clutch initiation date in our population.

In contrast to EPP, we observed a tendency for males with older social partners to lose paternity, independent of their own age, clutch initiation date or body size (tarsus length). Relatively few studies have reported a correlation between female age and occurrence of EPP, and these have provided inconsistent results, with some showing younger females of some species having higher levels of EPP (Stutchbury et al. 1997; Moreno et al. 2015) and others showing the reverse (Kempenaers et al. 1999; Whittingham and Dunn 2010). In other cases, there was no detectable correlation between female age and occurrence of extra-pair young (Lubjuhn et al. 2007; Costanzo et al. 2017a). Studies on barn swallows rarely report the effect of female age on paternity patterns, with two available studies detecting no effect (Møller et al. 2009a; Costanzo et al. 2017a). Given the scarcity of available data, we can only speculate that the discrepancy between these studies and our findings is due to differences in methodology (such as estimation of female ages), local differences in population structure or other reasons.

Interpretation of our results depends on the overall view of EPP in birds, with a continuing debate over the evolutionary origins and maintenance of extra-pair mating behaviour (e.g. Arnqvist and Kirkpatrick 2005; Albrecht et al. 2006; Eliassen and Kokko 2008; Forstmeier et al. 2014). If EPP evolved mainly as a female strategy, a higher occurrence of EPP in nests of older females could be interpreted as resulting from older and more experienced females being better able to evade mate guarding tactics, and hence be more likely to have multiple-sired broods (Dietrich et al. 2004; Bouwman and Komdeur 2005). It is also possible that more experienced (older) females are better at finding extra-pair sires. This should be especially noticeable in females paired with unattractive mates, such as young males (e.g. Dietrich et al. 2004; Ramos et al. 2014). In our study population, however, male age was not associated with paternity loss.

An alternative explanation for increased EPP in the nests of older females that does not explicitly assume that EPP is beneficial to females could be that older females are more attractive to males as female age is associated with traits considered attractive to males, such as phenotype, behavioural traits or body condition. Older females of most bird species tend to have higher clutch sizes and may produce higher-quality offspring than inexperienced breeders (e.g. Cichon 2003; Turner 2006; Decker et al. 2012). As extra-pair mate fecundity is a component of male fitness (Webster et al. 1995), older females could be chosen preferentially as EPP partners and coerced by other males to copulate outside their social pair bond.

Phenotypic traits previously identified as important determinants of male attractiveness in barn swallows, such as tail streamer length or ventral colouration (e.g. Møller 1994; Saino et al. 1997; Safran and McGraw 2004; Vortman et al. 2011; Hasegawa et al. 2012), were not significantly associated with male extra-pair and within-pair paternity success in our population. There may be a geographic variation in sexual selection on male ornament (Scordato and Safran 2014; Romano et al. 2017) and the information content of exaggerated male traits may differ in different environments. As a result, the reliability of an ornament signalling male quality may differ between barn swallow subspecies (Safran et al. 2016; Vitousek et al. 2016; Romano et al. 2017) and populations of the same subspecies may differ in traits associated with male attractiveness. This has previously been confirmed for North American barn swallows *H. r. erythrogaster* (Safran et al. 2005; Kleven et al. 2006a; Lifjeld et al. 2011).

In conclusion, our results suggest that male ornamentation per se contributes relatively little to overall variation in EPP compared to male age. As in some other bird populations (Hsu et al. 2015, 2017), therefore, age appeared to represent an important factor explaining EPP patterns in our barn swallow population. For the first time in barn swallows, our study also indicated that female age is associated with WPP loss. However, interpretation of our correlative results depends largely on differing opinions as to the evolutionary origin and maintenance of EPP in passerine populations (i.e. adaptive or non-adaptive scenarios; Forstmeier et al. 2014). It should also be noted that a large part of the observed variation (particularly in the case of within-pair fertilisation success) remained unexplained by our models. It would appear, therefore, that unmeasured factors, including genetic compatibility of social partners and self-referential, rather than absolute, criteria of mate choice (Mays and Hill 2004; Kempenaers 2007), may play an important role in determining the outcome of EPC in our barn swallow population.

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Author contributions TA conceived and designed the study; MA and OT undertook ornament analysis; RM, TA, OT, MA and JK performed the fieldwork; RM and JK conducted paternity analysis. TA and RM carried out the statistical analysis; RM and TA wrote the manuscript. All co-authors contributed to the final version of the manuscript and gave approval for its publication.

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Data availability The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standards This study complied with all applicable international, national and/or institutional guidelines on the use of animals. All protocols were non-invasive and adhered to the laws and guidelines of the Czech Republic (Czech Research Permit Numbers 6628/2008-10001). All protocols were approved by the Animal Care and Use Committees at the Czech Academy of Sciences (041/2011) and Charles University in Prague (4789/2008-30).

References

Akçay E, Roughgarden J (2007) Extra-pair paternity in birds: review of the genetic benefits. Evol Ecol Res 9:855–868

Albrecht T, Kreisinger J, Pialek J (2006) The strength of direct selection against female promiscuity is associated with rates of extrapair fertilizations in socially monogamous songbirds. Am Nat 167:739–744

Albrecht T, Schnitzer J, Kreisinger J, Exnerova A, Bryja J, Munclinger P (2007) Extra pair paternity and the opportunity for sexual selection in long-distant migratory passerines. Behav Ecol 18:477–486

Arnqvist G, Kirkpatrick M (2005) The evolution of infidelity in socially monogamous passerines: the strength of direct and indirect selection on extrapair copulation behavior in females. Am Nat 165:26–37

Balbontín J, Hermosell IG, Marzal A, Reviriego M, De Lope F, Møller AP (2007) Age-related change in breeding performance in early life is associated with an increase in competence in the migratory barn swallow *Hirundo rustica*. J Anim Ecol 76:915–925

Balbontín J, Møller AP, Hermosell IG, Marzal A, Reviriego M (2012) Geographical variation in reproductive ageing patterns and life-history strategy of a short-lived passerine bird. J Evol Biol 25: 2298–2309

Bartoń K (2016) MuMIn: multi-model inference. R package version 1.15.6, https://CRAN.R-project.org/package=MuMIn

Bates D, Maechler M, Bolker BM, Walker SC (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48

Birkhead TR (2010) How stupid not to have thought of that: post-copulatory sexual selection. J Zool 281:78–93

Bitton PP, O'Brien EL, Dawson RD (2007) Plumage brightness and age predict extrapair fertilization success of male tree swallows, *Tachycineta bicolor*. Anim Behav 74:1777–1784

Bouwman KM, Komdeur J (2005) Old female reed buntings (*Emberiza schoeniclus*) increase extra-pair paternity in their broods when mated to young males. Behaviour 142:1449–1463

Bouwman KM, van Dijk RE, Wijmenga JJ, Komdeur J (2007) Older male reed buntings are more successful at gaining extrapair fertilizations. Anim Behav 73:15–27

Bowers EK, Forsman AM, Masters BS, Johnson BGP, Johnson LS, Sakaluk SK, Thompson CF (2015) Increased extra-pair paternity in broods of aging males and enhanced recruitment of extra-pair young in a migratory bird. Evolution 69:2533–2541

Brooks R, Kemp DJ (2001) Can older males deliver the good genes? Trends Ecol Evol 16:308–313

Bryja J, Patzenhauerova H, Albrecht T, Mosansky L, Stanko M, Stopka P (2008) Varying levels of female promiscuity in four *Apodemus* mice species. Behav Ecol Sociobiol 63:251–260

Canal D, Potti J, Davila JA (2011) Male phenotype predicts extra-pair paternity in pied flycatchers. Behaviour 148:691–712

Cichon M (2003) Does prior breeding experience improve reproductive success in collared flycatcher females? Oecologia 134:78–81

Coleman SW, Jones AG (2011) Patterns of multiple paternity and maternity in fishes. Biol J Linn Soc 103:735–760

Costanzo A, Ambrosini R, Capriolo M, Gatti E, Parolini M, Romano A, Rubolini D, Gianfranceschi L, Saino N (2017a) Extrapair fertilizations vary with female traits and pair compositions, besides male attractiveness, in barn swallows. Anim Behav 134:183–191

Costanzo A, Ambrosini R, Caprioli M, Gatti E, Parolini M, Canova L, Rubolini D, Romano A, Gianfranceschi L, Saino N (2017b) Lifetime reproductive success, selection on lifespan, and multiple sexual ornaments in male European barn swallows. Evolution 71: 2457–2468

Dakin EE, Avise JC (2004) Microsatellite null alleles in parentage analysis. Heredity 93:504–509

Decker KL, Conway CJ, Fontaine JJ (2012) Nest predation, food, and female age explain seasonal declines in clutch size. Evol Ecol 26: 683–699

Dietrich V, Schmoll T, Winkel W, Epplen JT, Lubjuhn T (2004) Pair identity—an important factor concerning variation in extra-pair paternity in the coal tit (*Parus ater*). Behaviour 141:817–835

Edme A, Munclinger P, Krist M (2016) Female collared flycatchers choose neighbouring and older extra-pair partners from the pool of males around their nests. J Avian Biol 47:552–562

Eliassen S, Kokko H (2008) Current analyses do not resolve whether extra-pair paternity is male or female driven. Behav Ecol Sociobiol 62:1795–1804

Endler JA, Mielke PW (2005) Comparing entire colour patterns as birds see them. Biol J Linn Soc 86:405–431

Foerster K, Delhey K, Johnsen A, Lifjeld JT, Kempenaers B (2003) Females increase offspring heterozygosity and fitness through extra-pair matings. Nature 425:714–717

Forstmeier W, Nakagawa S, Griffith SC, Kempenaers B (2014) Female extra-pair mating: adaptation or genetic constraint? Trends Ecol Evol 29:456–464

Freeman-Gallant CR, Taff CC, Morin DF, Dunn PO, Whittingham LA, Tsang SM (2010) Sexual selection, multiple male ornaments, and age- and condition-dependent signalling in the common yellowthroat. Evolution 64:1007–1017

Froy H, Phillips RA, Wood AG, Nussey DH, Lewis S (2013) Age-related variation in reproductive traits in the wandering albatross: evidence for terminal improvement following senescence. Ecol Lett 16:642–649

Goldsmith TH (1990) Optimization, constraint, and history in the evolution of eyes. Q Rev Biol 65:281–322

Griffith SC, Owens IPF, Thuman KA (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. Mol Ecol 11:2195–2212

Grunst AS, Grunst ML (2014) Multiple sexual pigments, assortative social pairing, and genetic paternity in the yellow warbler (*Setophaga petechia*). Behav Ecol Sociobiol 68:1451–1463

Hasegawa M, Arai E, Watanabe M, Nakamura M (2010) Mating advantage of multiple male ornaments in the barn swallow *Hirundo rustica gutturalis*. Ornithol Sci 9:141–148

Hasegawa M, Arai E, Watanabe M, Nakamura M (2012) High incubation investment of females paired to attractive males in barn swallows. Ornithol Sci 11:1–8

Hsu YH, Schroeder J, Winney I, Burke T, Nakagawa S (2015) Are extra-pair males different from cuckolded males? A case study and a meta-analytic examination. Mol Ecol 24:1558–1571

Hsu YH, Simons MJP, Schroeder J, Girndt A, Winney IS, Burke T, Nakagawa S (2017) Age-dependent trajectories differ between within-pair and extra-pair paternity success. J Evol Biol 30:951–959

Hubbard JK, Jenkins BR, Safran RJ (2015) Quantitative genetics of plumage color: lifetime effects of early nest environment on a colorful sexual signal. Ecol Evol 5:3436–3449

Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour 10:551–555

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099–1106

Kempenaers B (2007) Mate choice and genetic quality: a review of the heterozygosity theory. Adv Study Behav 37:189–278

Kempenaers B, Verheyen G, Vandenbroeck M, Burke T, Van Broeckhoven C, Dhondt A (1992) Extra-pair paternity results from female preference for high-quality males in the blue tit. Nature 357: 494–496

Kempenaers B, Verheyen GR, Dhondt AA (1997) Extrapair paternity in the blue tit (*Parus caeruleus*): female choice, male characteristics, and offspring quality. Behav Ecol 8:481–492

Kempenaers B, Congdon B, Boag P, Robertson RJ (1999) Extrapair paternity and egg hatchability in tree swallows: evidence for the genetic compatibility hypothesis? Behav Ecol 10:304–311

Kleven O, Jacobsen F, Izadnegahdar R, Robertson RJ, Lifjeld JT (2006a) Male tail streamer length predicts fertilization success in the North American barn swallow (*Hirundo rustica erythrogaster*). Behav Ecol Sociobiol 59:412–418

Kleven O, Marthinsen G, Lifjeld JT (2006b) Male extraterritorial forays, age and paternity in the socially monogamous reed bunting (*Emberiza schoeniclus*). J Ornithol 147:468–473

Kokko H (1998) Good genes, old age and life-history trade-offs. Evol Ecol 12:739–750

Kose M, Mand R, Møller AP (1999) Sexual selection for white tail spots in the barn swallow in relation to habitat choice by feather lice. Anim Behav 58:1201–1205

Kreisinger J, Čížková D, Kropáčková L, Albrecht T (2015) Cloacal microbiome structure in a long-distance migratory bird assessed using deep 16sRNA pyrosequencing. PLoS One 9:e0137401

Kruuk LEB, Merilä J, Sheldon BC (2001) Phenotypic selection on a heritable size trait revisited. Am Nat 158:557–571

Lifjeld JT, Kleven O, Jacobsen F, McGraw KJ, Safran RJ, Robertson RJ (2011) Age before beauty? Relationships between fertilization success and age-dependent ornaments in barn swallows. Behav Ecol Sociobiol 65:1687–1697

Lubjuhn T, Gerken T, Bruen J, Schmoll T (2007) Yearling male great tits, *Parus major*, suffer more strongly from cuckoldry than older males. Zoology 110:387–397

Lyu N, Servedio MR, Sun YH (2018) Nonadaptive female pursuit of extrapair copulations can evolve through hitchhiking. Ecol Evol 8: 3685–3692

Maia R, Eliason CM, Bitton PP, Doucet SM, Shawkey MD (2013) pavo: an R package for the analysis, visualization and organization of spectral data. Methods Ecol Evol 4:906–913

Manning JT (1985) Choosy females and correlates of male age. J Theor Biol 116:349–354

Mauck RA, Huntington CE, Grubb TC (2004) Age-specific reproductive success: evidence for the selection hypothesis. Evolution 58:880–885

Mays HL, Hill GE (2004) Choosing mates: good genes versus genes that are a good fit. Trends Ecol Evol 19:554–559

Mays HL, Albrecht T, Liu M, Hill GE (2008) Female choice for genetic complementarity in birds: a review. Genetica 134:147–158

Møller AP (1994) Sexual selection and the barn swallow. Oxford University Press, Oxford

Møller AP (2017) Experimental manipulation of size and shape of tail spots and sexual selection in barn swallows. Curr Zool 63:569–572

Møller AP, de Lope F (1999) Senescence in a short-lived migratory bird: age-dependent morphology, migration, reproduction and parasitism. J Anim Ecol 68:163–171

Møller AP, Brohede J, Cuervo JJ, de Lope F, Primmer C (2003) Extrapair paternity in relation to sexual ornamentation, arrival date, and condition in a migratory bird. Behav Ecol 14:707–712

Møller AP, Balbontín J, Cuervo JJ, Hermosell IG, de Lope F (2009a) Individual differences in protandry, sexual selection, and fitness. Behav Ecol 20:433–440

Møller AP, Mousseau TA, Rudolfsen G, Balbontín J, Marzal A, Hermosell I, de Lope F (2009b) Senescent sperm performance in old male birds. J Evol Biol 22:334–344

Moreno J, Martinez JG, Gonzalez-Braojos S, Cantarero A, Ruiz-de-Castaneda R, Precioso M, Lopez-Arrabe J (2015) Extra-pair paternity declines with female age and wing length in the pied flycatcher. Ethology 121:501–512

Ninni P (2003) Carotenoid signals in barn swallows. PhD thesis, Université Pierrre et Marie Curie, Paris, France

O'Brien EL, Dawson RD (2011) Plumage color and food availability affect male reproductive success in a socially monogamous bird. Behav Ecol 22:66–72

Petrželková A, Michálková R, Albrechtová J, Cepák J, Honza M, Kreisinger J, Munclinger P, Soudková M, Tomášek O, Albrecht T (2015) Brood parasitism and quasi-parasitism in the European barn swallow *Hirundo rustica rustica*. Behav Ecol Sociobiol 69:1405–1414

Ramos AG, Nunziata SO, Lance SL, Rodriguez C, Faircloth BC, Gowaty PA, Drummond H (2014) Interactive effects of male and female age on extra-pair paternity in a socially monogamous seabird. Behav Ecol Sociobiol 68:1603–1609

Richardson DS, Burke T (1999) Extra-pair paternity in relation to male age in Bullock's orioles. Mol Ecol 8:2115–2126

Romano A, Costanzo A, Rubolini D, Saino N, Møller AP (2017) Geographical and seasonal variation in the intensity of sexual selection in the barn swallow *Hirundo rustica*:a meta-analysis. Biol Rev 92:1582–1600

Safran RJ, McGraw KJ (2004) Plumage coloration, not length or symmetry of tail-streamers, is a sexually selected trait in North American barn swallows. Behav Ecol 15:455–461

Safran RJ, Neuman CR, McGraw KJ, Lovette IJ (2005) Dynamic paternity allocation as a function of male plumage color in barn swallows. Science 309:2210–2212

Safran RJ, Vortman Y, Jenkins BR, Hubbard JK, Wilkins MR, Bradley RJ, Lotem A (2016) The maintenance of phenotypic divergence through sexual selection: an experimental study in barn swallows *Hirundo rustica*. Evolution 70:2074–2084

Saino N, Primmer CR, Ellegren H, Møller AP (1997) An experimental study of paternity and tail ornamentation in the barn swallow (*Hirundo rustica*). Evolution 51:562–570

Saino N, Romano M, Rubolini D, Ambrosini R, Caprioli M, Milzani A, Costanzo A, Colombo G, Canova L, Wakamatsu K (2013) Viability is associated with melanin-based coloration in the barn swallow (*Hirundo rustica*). PLoS One 8:e60426

Scordato ESC, Safran RJ (2014) Geographic variation in sexual selection and implications for speciation in the barn swallow. Avian Res 5:8

Stoddard MC, Prum RO (2008) Evolution of avian plumage color in a tetrahedral color sdpace: a phylogenetic analysis of New World buntings. Am Nat 171:755–776

Stoffel MA, Nakagawa S, Schielzeth H (2017) rptR: repeatability estimation and variance decomposition by generalized mixed-effects models. Methods Ecol Evol 8:1639–1644

Stutchbury BJM, Piper WH, Neudorf DL, Tarof SA, Rhymer JM, Fuller G, Fleischer RC (1997) Correlates of extra-pair fertilization success in hooded warblers. Behav Ecol Sociobiol 40:119–126

Turner A (2006) The barn swallow. T & AD Poyser, London

Uller T, Olsson M (2008) Multiple paternity in reptiles: patterns and processes. Mol Ecol 17:2566–2580

Vitousek MN, Tomášek O, Albrecht T, Wilkins MR, Safran RJ (2016) Signal traits and oxidative stress: a comparative study across populations with divergent signals. Front Ecol Evol 4:56

Vortman Y, Lotem A, Dor R, Lovette IJ, Safran RJ (2011) The sexual signals of the East-Mediterranean barn swallow: a different swallow tale. Behav Ecol 22:1344–1352

Wang JL (2004) Sibship reconstruction from genetic data with typing errors. Genetics 166:1963–1979

Webster MS, Pruett-Jones S, Westneat DF, Arnold SJ (1995) Measuring the effects of pairing success, extra-pair copulations and mate quality on the opportunity for sexual selection. Evolution 49:1147–1157

Webster MS, Tarvin KA, Tuttle EM, Pruett-Jones S (2007) Promiscuity drives sexual selection in a socially monogamous bird. Evolution 61:2205–2211

Westneat DF, Stewart IRK (2003) Extra-pair paternity in birds: causes, correlates, and conflict. Annu Rev Ecol Evol Syst 34:365–396

Whittingham LA, Dunn PO (2010) Fitness benefits of polyandry for experienced females. Mol Ecol 19:2328–2335

Zuur AF, Ieno EN, Elphick CS (2010) Protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14

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Behavioral Ecology and Socobiology

Electronic Supplementary Information

for

Extra-pair paternity patterns in European barn swallows *Hirundo rustica rustica* are best explained by male and female age rather than male ornamentation

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Supplementary inventory

Supplementary Table S1 Summary statistics for microsatellite loci used to determine paternity in barn swallows

Supplementary Table S2 Prevalence of extra-pair paternity and extra-pair young

Supplementary Table S3 Results of univariate analyses of the relationship between clutch initiation date and selected male phenotypes and male and female age

Supplementary Table S4 Results of univariate analyses of the relationship between selected male phenotypes and age

Supplementary Table S5 Results of univariate analyses of male extra-pair fertilisation success in relation to male phenotypes and the timing of breeding

Supplementary Table S6 Results of univariate analyses of number of extra-pair young sired out of the male social nest in relation to male phenotypes and the timing of breeding

Supplementary Table S7 Results of univariate analyses of male within-pair fertilization success in relation to male phenotypes, the timing of breeding and social partner age

Supplementary Table S8 Results of univariate analyses of proportion of within-pair and extra-pair young in the social nest in relation to male phenotypes, male and female age and the timing of breeding

Supplementary Table S1 Summary statistics for microsatellite loci used to determine paternity in barn swallows. Locus – name of locus described by Primmer et al. 1996 or Tsyusko et al. 2007; N – number of typed individual; K – number of alleles; H(obs) – observed heterozygosity; H(exp) – expected heterozygosity; PIC – polymorphic information content; F(Null) – frequency of null alleles, labelling – dye which was used for each forward primer

Locus	K	N	H(obs)	H(exp)	PIC	F(Null)	labelling
Hir15	12	2277	0.652	0.682	0.627	0.023	FAM
Hir10	15	2277	0.788	0.835	0.815	0.027	HEX
Hir20	22	2275	0.826	0.843	0.824	0.009	HEX
Hir6	18	2276	0.857	0.842	0.822	-0.009	NED
Hir22	19	2277	0.829	0.876	0.863	0.026	NED
HrU10	49	2274	0.95	0.952	0.948	0.036	PET

Supplementary Table S2 Prevalence of extra-pair paternity (EPP) and extra-pair young (EPY) in a South Bohemian (Czech Republic) barn swallow population during the years 2010 – 2013

Year	Nests	EPP nests (%)	Young	EPY (%)
2010	13	7 (53%)	57	16 (28%)
2011	32	13 (40%)	138	20 (14.5%)
2012	48	24 (50%)	214	51 (23.8%)
2013	67	38 (56%)	285	51 (17.8%)
Total:	160	82 (51.2%)	694	138 (19.8%)

Supplemetary Table S3 Predictors of clutch initiation date (standardized across years). Shown are results of univariate analyses of the relationship between clutch initiation date and selected male phenotypes and male and female age (n = 160). Estimates are based on GLMM model (male identity as a random grouping factor). Explanatory variables associated significantly with clutch initition date are indicated bold; nlme package (Pinheiro et al. 2018) was used for calculations

	Value	Std. Error	t value	p value
Tail streamer length	-0.133	0.081	-1.643	0.107
Tail spots	-0.035	0.081	-0.432	0.668
Ventral θ	**0.295**	**0.075**	**3.901**	**< 0.001**
Throat θ	0.126	0.077	1.626	0.111
Throat φ	-0.051	0.076	-0.664	0.510
Tarsus length	-0.052	0.078	-0.662	0.511
Male age	**-0.428**	**0.071**	**-6.042**	**< 0.001**
Female age	**-0.334**	**0.073**	**-4.562**	**< 0.001**

Supplemetary Table S4 Results of univariate analyses of the relationship between selected male phenotypes and male age (n = 160). Estimates are based on GLMM model (male identity as a random grouping factor) with age as a continous variable. Explanatory variables associated significantly with male age are indicated bold; nlme package (Pinheiro et al. 2018) was used for calculations

	Value	Std. Error	t value	p value
Tail streamer length	**0.283**	**0.065**	**4.337**	**< 0.001**
Tail spots	**0.285**	**0.079**	**3.616**	**< 0.001**
Ventral θ	-0.116	0.085	-1.361	0.181
Throat θ	-0.171	0.086	-1.987	0.053
Throat φ	0.06	0.085	0.703	0.486
Tarsus length	0.084	0.086	0.967	0.339
Clutch initiation date	**-0.361**	**0.067**	**-5.347**	**< 0.001**

Supplementary Table S5 Results of univariate analyses of male extra-pair fertilisation success in relation to male phenotypes and the timing of breeding (n = 160). Estimates are based on GLMM model (male identity and year-locality as random grouping factors) with binary response variable (1 – at least one offspring sired extrapair, 0 – no offspring sired extrapair) and logit-link function. Predictors were standardized (z-transformed) prior to analysis. Explanatory variables associated significantly with male extra-pair fertilization success are indicated bold

	AIC	Estimate	Std. Error	z value	p value
Tail streamer length	223.3	0.331	0.172	1.925	0.054
Tail spots	224.4	0.288	0.181	1.595	0.111
Ventral θ	226.4	-0.147	0.172	-0.854	0.393
Throat θ	226.7	-0.102	0.172	-0.597	0.551
Throat φ	226.8	0.091	0.173	0.524	0.6
Tarsus length	226.7	0.105	0.167	0.628	0.53
Clutch initiation date	**213.3**	**-0.661**	**0.189**	**-3.49**	**< 0.001**
Male age	**198.9**	**1.007**	**0.217**	**4.635**	**< 0.001**

Supplementary Table S6 Results of univariate analyses of number of extra-pair young sired out of the male social nest in relation to male phenotypes and the timing of breeding (n = 160). Estimates are based on GLMM model (male identity and year-locality as random grouping factors) with number of EPY as a continous variable and Poisson error distribution in R (log link). Predictors were standardized (z-transformed) prior to analysis. Explanatory variables associated significantly with number of EPY sired are indicated bold

	AIC	Estimate	Std. Error	z value	p value
Tail streamer length	473.1	0.17	0.112	1.516	0.129
Tail spots	471.7	0.211	0.111	1.896	0.058
Ventral θ	475.4	-0.017	0.106	-0.163	0.871
Throat θ	475	0.061	0.098	0.627	0.53
Throat φ	475.1	-0.05	0.091	-0.551	0.582
Tarsus length	**471.2**	**0.212**	**0.099**	**2.124**	**0.034**
Clutch initiation date	**459.6**	**-0.42**	**0.105**	**-3.991**	**< 0.001**
Male age	**450.6**	**0.493**	**0.102**	**4.833**	**< 0.001**

Supplementary Table S7 Results of univariate analyses of male within-pair fertilisation success in relation to male phenotypes, the timing of breeding and social partner age (n = 160). Estimates are based on GLMM model (male identity and year-locality as random grouping factors) with binary response variable (1 – at least one offspring sired extrapair, 0 – no offspring sired extrapair) and logit-link function. Predictors were standardized (z-transformed) prior to analysis. Explanatory variable associated significantly with male within-pairfertilization success is indicated bold

	AIC	Estimate	Std. Error	z value	p value
Tail streamer length	227.6	0.223	0.184	1.23	0.219
Tail spots	227.4	0.242	0.184	1.313	0.189
Ventral θ	229	0.077	0.176	0.436	0.663
Throat θ	229.2	-0.015	0.173	-0.088	0.93
Throat φ	229.1	-0.079	0.175	-0.45	0.653
Tarsus length	227.8	0.209	0.181	1.155	0.248
Clutch initiation date	228.6	-0.14	0.18	-0.778	0.437
Male age	227.6	0.235	0.198	1.186	0.236
Female age	**220.4**	**0.556**	**0.206**	**2.696**	**0.007**

Supplementary Table S8 Results of univariate analyses of proportion of within-pair and extra-pair young in the social nest in relation to male phenotypes, male and female age and the timing of breeding (n = 160 nests). Estimates are based on GLMM model (male and female identity and year-locality as random grouping factors) with proportion response variable (cbind(WPY/EPY)) and logit-link function. Predictors were standardized (z-transformed) prior to analysis. Explanatory variable associated significantly with proportion of WPY/total young is indicated bold

	AIC	Estimate	Std. Error	*z* value	*p* value
Tail streamer length	398	0.098	0.119	0.824	0.41
Tail spots	398	0.097	0.119	0.817	0.414
Ventral θ	397.5	0.128	0.118	1.083	0.279
Throat θ	398.7	-0.004	0.114	-0.038	0.97
Throat φ	398.5	0.047	0.112	0.419	0.675
Tarsus length	398.2	0.083	0.119	0.697	0.486
Clutch initiation date	397.8	-0.122	0.119	-1.027	0.304
Male age	398.5	0.074	0.122	0.605	0.545
Female age	**392.6**	**0.309**	**0.123**	**2.505**	**0.012**

Function 1. (source: https://stats.stackexchange.com/questions/201205/variance-inflation-factors-vif-mer-verus-group-factor-level-vif). Vif.mer function to estimate collinearity between predictors of mixed effect models

```
vif.mer <- function (fit) {
v <- vcov(fit)
nam <- names(fixef(fit))
ns <- sum(1 * (nam == "Intercept" | nam == "(Intercept)"))
if (ns > 0) {
v <- v[-(1:ns), -(1:ns), drop = FALSE]
nam <- nam[-(1:ns)] }
d <- diag(v)^0.5
v <- diag(solve(v/(d %o% d)))
names(v) <- nam v }
```

APENDIX

CURRICULUM VITAE

Vzdělání:

2012-present:	Ph.D. student – Zoologie
	Univerzita Karlova v Praze, Přírodovědecká fakulta, Katedra Zoologie, Praha
2009-2012:	Mgr. student – Zoologie
	Univerzita Karlova v Praze, Přírodovědecká fakulta, Katedra Zoologie, Praha
2006-2009:	Bc. student – Biologie
	Univerzita Karlova v Praze, Přírodovědecká fakulta, studijní program: Biologie, Praha

Pracovní zkušenosti:

2017-současnost:	Klinický bioanalytik a výzkumný pracovník, Ústav patologie 1. LF UK a VFN, Praha
2019-současnost:	Vědecký pracovník, laborant, Ústav biologie obratlovců AV ČR, v. v. i
2013-2018:	Vědecký pracovník, laborant, Přírodovědecká fakulta, Katedra Zoologie, Oddělení evoluční biologie živočichů, Praha

Zkušenosti s vyučováním:

2012-současnost:	Univerzita Karlova v Praze, Přírodovědecká fakulta, Praha
	Morfologie živočichů (praktická výuka)
	Zoologie obratlovců (praktická výuka)
	Školitel Bc. studenta (1 dokončený)

Účast na projektech:

2013-2015:	Alternativní reprodukční strategie: evoluční význam parazitického a promiskuitního chování samic u sociálně monogamního pěvce (Grantová agentura Univerzity Karlovy, GA UK 146213, spolu-řešitel)
2012-2016:	Post-copulatory sexual selection and the biology of sperm: within population processes and interspecific patterns in birds (Grantová agentura České republiky, GA ČR, P506-12-2472, spolu-řešitel)

Zahraniční spolupráce:

- Dr. Rebecca J. Safran, Ecology and Evolutionary Biology, University of Colorado, Boulder
- Dr. Péter L. Pap, Evolutionary Ecology Group, Babeş-Bolyai University, Cluj Napoca, Rumunsko

Vědecké zájmy:

- alternativní reprodukční strategie, pohlavní výběr, evoluce promiskuity u ptáků
- molekulární patologie rakoviny, mutační analýza

VYBRANÉ PUBLIKACE

Publikace, které jsou součástí disertační práce:

1. **Michálková R***., Petrželková A.*, Albrechtová J., Cepák J., Honza M., Kreisinger J., Munclinger P., Soudková M., Tomášek O. & Albrecht T. (2015). Brood parasitism and quasi-parasitism in the European barn swallow *Hirundo rustica rustica*. *Behavioral Ecology and Sociobiology* 69: 1405-1414.

   (* R. Michálková & A. Petrželková contributed equally to this work)

2. Tomášek O., Pap P. L., Adámková M., Cepák J., Fulop A., **Michálková R**., Stermin A. N., Vágási C. I., Vincze O. & Albrecht T. Age-dependence and viability signaling function of tail streamer length in the European barn swallow (*in preparation for Journal of Animal Ecology*).

3. Pap P. L., Fülöp A., Adámková M., Cepák J., **Michálková R**., Safran R. J., Stermin A. N., Tomášek O., Vágási C. I., Vincze O., Wilkins M. R. & Albrecht T. (2019). Selection on multiple sexual signals in two Central and Eastern European populations of the barn swallow. *Ecology and Evolution* 9: 11277-11287.

4. **Michálková R**., Tomášek O., Adámková M., Kreisinger J. & Albrecht T. (2019). Extra-pair paternity patterns in European barn swallows *Hirundo rustica* are best explained by male and female age rather than male ornamentation. *Behavioral Ecology and Sociobiology* 73: 119.

Ostatní publikace, týkající se výzkumu na vlaštovce obecné:

Kreisinger J., Kropáčková L., Petrželková A., Adámková M., Tomášek O., Martin J., **Michálková R**. & Albrecht T. (2017). Temporal Stability and the Effect of Transgenerational Transfer on Fecal Microbiota structure in a Long Distance Migratory bird. *Frontiers in Microbiology* 8:50.

Kreisinger J., Schmiedová L., Petrželková A., Tomášek O., Adámková M., **Michálková R**., Martin J. & Albrecht T. (2018). Fecal microbiota associated with phytohaemagglutinin-induced immune response in nestlings of a passerine bird. *Ecology and Evolution* 20;8(19): 93793-9802.

Klvaňa P., Cepák J., Munclinger P., **Michálková R**., Tomášek O. & Albrecht T. (2018). Around the Mediterranean: an extreme example of loop migration in a long-distance migratory passerine. *Journal of Avian Biology* 49: jav-01595.

Jiné publikace (patologický výzkum):

Hojný J., **Michálková R**., Krkavcová E., Quang Hiep B., Bártů M., Němejcová K., Kalousová M., Kleiblová P., Dundr P. & Stružinská I. (2021). Comprehensive quantitative analysis of known and novel alternative splicing variants completed HNF1B mRNA splicing pattern in various tumour and non-tumour tissues. *International Journal of Molecular Sciences - under review status for a minor revision.*

Němejcová K., Bártů M., Hojný J., Hájková N., **Michálková R**., Krkavcová E., Stružinská I., Quang Bui H., Dundr P., Cibula D. & Jirsová K. (2021). A comprehensive analysis of the expression, epigenetic and genetic changes of HNF1B and ECI2 in 122 cases of high-grade serous ovarian carcinoma. *Oncology letters* 3:185.

Dundr P., Bártů M., Hojný J., **Michálková R.**, Hájková N., Stružinská I., Krkavcová E., Hadravský L., Kleissnerová L., Kopejsková J., Quang Hiep B., Němejcová K., Jakša R., Čapoun O., Řezáč J., Jirsová K. & Franková V. (2020). HNF1B, EZH2 and ECI2 in prostate carcinoma. Molecular, immunohistochemical and clinico-pathological study. *Scientific Reports* 1;10(1):14365.

Bártů M., Hojný J., Hájková N., **Michálková R.**, Krkavcová E., Simon K., Frýba V., Stružinská I., Němejcová K. & Dundr P. (2020). Expression, epigenetic, and genetic changes of HNF1B in colorectal lesions: an analysis of 145 cases. *Pathology and Oncology Research* 2020; doi:10.1007/s12253-020-00830-2.

Tichá I., Hojný J., **Michálková R.**, Kodet O., Krkavcová E., Hájková N., Němejcová K., Bartů M., Jakša R., Důra M., Kanwal M., Martiníková A.S., Macurek L., Zemánková P., Kleibl Z. & Dundr P. (2019). A comprehensive evaluation of pathogenic mutations in primary cutaneous melanomas, including the identification of novel loss-function variants. *Scientific Reports* 9: 17050.

Němejcová K., Tichá I., Bártů M., Kodet O., Důra M., Jakša R., **Michálková R**. & Dundr P (2019). Comparison of five different scoring methods in the evaluation of inflammatory infiltration (Tumor-Infiltrating Lymphocytes) in superficial spreading and nodular melanoma. *Pigment Cell Melanoma Research* 32: 412-423.

Dundr P., Simon K., Němejcová K., Bártů M., Tichá I., **Michálková R**., Jakša R., Věcková Z. & Kodet O. (2019). Stathmin is a potential therapeutic target but not prognostic marker in melanoma. An immunohistochemical study of 323 melanocytic lesions. *Melanoma Research* 29: 157-162.

Hájková N., Tichá I., Hojný J., Němejcová K., Bártů M., **Michálková R**., Zikán M., Cibula D., Laco J., Geryk T., Méhes G. & Dundr P. (2019). Synchronous endometrioid endometrial and ovarian carcinomas are biologically related: A clinico-pathological and molecular (next generation sequencing) study of 22 cases. *Oncology letters* 17: 2207-2214.

Důra M., Němejcová K., Jakša R., Bártů M., Kodet O., Tichá I., **Michálková R**. & Dundr P. (2019). Expression of Glut-1 in malignant melanoma and melanocytic nevi: An immunohistochemical study of 400 cases. *Pathology and Oncology Research* 25: 361-368.